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Official Journal of The Endocrine Society

VOLUME 12 NUMBER 7

JULY, 1952

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VOLUME 12 JULY, 1952 NUMBER 7
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THE USE OF INTRAVENOUS ACTH: A STUDY IN QUANTITATIVE ADRENOCORTICAL STIMULATION*

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IN THE widely used method of Sayers *et al.* (1) for the assay of ACTH in rats, using the adrenal depletion of ascorbic acid as an index of adrenal cortical activation, the material is given intravenously. More reliable results are obtained by that route of administration than by intramuscular injection. Ingle (2) was the first to demonstrate the value of continuous subcutaneous administration of small quantities of ACTH in producing maximal hypertrophy of the adrenal cortices of rats. After two

Received for publication March 4, 1952.

* Presented in part at the Annual Meeting of the Association for the Study of Internal Secretions, Atlantic City, N. J., June 7–9, 1951.

This study was aided in part by grants from the National Institutes of Health, United States Public Health Service; the Swiss Academy of Medical Sciences; the Armour Laboratories, Chicago, Illinois; and the Higgins Fund of Harvard Medical School.

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to three days the size of the gland increased to an extent never observed when the hormone was administered in separate injections.

Sayers *et al.* (3) were the first to administer ACTH intravenously in man. Large doses, given over thirty minutes to 2 normal subjects, produced most of the known physiologic effects in spite of the rapid disappearance of ACTH from the blood stream following the end of the infusion. In clinical usage the intramuscular route of administration remained the method of choice until Gordon (4) demonstrated the usefulness of the intravenous route in man, using a continuous infusion extending over a period of days. Gordon's findings, which have been amply confirmed and extended, established the intravenous use of ACTH as a clinically effective and economical method of inducing adrenal cortical stimulation (5, 6, 7, 8).

The intramuscular administration of ACTH does not lend itself to quantitative estimation of adrenal cortical activation because of the variability of local absorption of ACTH and because of the unpredictable amount of local destruction in the tissues (9, 10). For these reasons the intravenous infusion of the hormone has been selected in these pharmacologic studies of ACTH in man. In this paper the criteria of adrenal cortical activation are reviewed, the effect of dosage of ACTH and duration of administration are investigated, and a rational scheme for the intravenous use of ACTH in the diagnosis of adrenocortical dysfunction is outlined. A comparison of single, daily and continuous infusions of the hormone will be presented, the question of induced resistance to the action of certain types of ACTH will be discussed and the clinical usefulness of the mode of administration will be evaluated.

MATERIALS AND METHODS

The bulk of the study was carried out with Armour ACTH (ACTHAR), a lyophilized powder obtained from frozen hog pituitaries by acid-acetone extraction followed by isoelectric precipitation in the cold. Several different lots were used and ACTH preparations of similar nature prepared from beef and sheep pituitaries were also employed. In order to control the effects observed with these commercial preparations, tests were carried out with both a partly purified fraction C and a highly purified fraction D of the hormone as prepared by Dr. E. B. Astwood, using glacial acetic acid extraction of hog pituitaries, followed by purification on cellulose columns and differential precipitation (11). Both types of hormones were compared in a metabolic study with a highly purified preparation obtained by counter-current distribution of the peptic hydrolysate of ACTH as prepared by Lesh (12).

Subjects and patients were studied on the metabolic wards of the Peter Bent Brigham Hospital under controlled conditions.

Unless otherwise specified, ACTH was administered as a *continuous*

intravenous infusion of 20 I.U. (equivalent to 20 mg. of the Armour Standard LA-1-A) in 500 ml. of either physiologic salt solution or 5 per cent dextrose in water, over suitable periods of time. The solutions were always freshly prepared before administration, and at eight-hourly intervals whenever the duration of the infusion extended beyond this period. The use of 25-gauge ½-inch needles tightly taped to the forearm facilitated the procedure. For prolonged intravenous infusion a polyvinyl catheter (B. D. No. 442T) was introduced into the basilic or cephalic vein through an 18-gauge thin walled needle and inserted into the subclavian vein.[1] For *intramuscular infusions* the same preparations of ACTH were used and administered through a 23-gauge, 2-inch needle inserted into the anterior thigh musculature after previous infiltration with 1 per cent procaine solution. After an initial series of intraocular sensitivity tests which yielded negative results, subjects were no longer investigated for skin or ocular sensitivity to ACTH.

Chemical methods employed were those previously described (13, 14). In addition, formaldehydogenic steroids were determined by a modification of the method of Daughaday *et al.* (15) and 17-ketosteroids by a modified procedure based on standard methods (16, 17), which is presented here in its simplest form:

The urine is collected in 24-hour periods, with 3 ml. of 50 per cent sulfuric acid added as a preservative. A 50-ml. aliquot is hydrolyzed by refluxing for seven minutes with 7.5 ml. of concentrated HCl, then cooled in crushed ice. The hydrolyzed urine is then extracted in a 250-ml. separatory funnel, twice with 20 ml. and once with 10 ml. of ether, shaking ninety seconds each time. The ether extracts are combined and washed three times with 10 ml. of a freshly prepared solution of 10 per cent sodium hydrosulfite in 2.5 normal NaOH, and three times with 10 ml. of distilled water. The third water washing should be neutral to indicator paper. The ether extract is then transferred to a 125-ml. Erlenmeyer flask and evaporated to dryness over a warm electric hotplate under a continuous stream of air at reduced pressure. The ether should remain cool throughout and the hot plate is removed when only a few drops of fluid remain. The dried extract, which may be stored indefinitely, is then dissolved in 1 ml. of redistilled, ketone-free, absolute ethanol, heating the stoppered flasks in warm water to aid dissolution. The Zimmermann color reaction is then carried out, using 0.2-ml. samples of the unknown with two absolute alcohol blanks and three dehydroandrosterone standards (25 mg. of dehydroandrosterone in 100 ml. of absolute alcohol). A freshly prepared solution of purified, twice recrystallized metadinitrobenzene crystals (16), and KOH made from fresh pellets and stored under a layer of mineral oil for not over four weeks in the cold, are used.

PHARMACOLOGIC OBSERVATIONS

Indices of adrenal cortical activation

The quantitative evaluation of adrenocortical stimulation may be based on a number of indices. Ideally the hormonal output of the adrenal cortex

[1] We are indebted to Dr. Walter T. Goodale for carrying out this procedure.

could be measured directly by the determination of these hormones in the blood stream (18). Such a method was not available when this study was undertaken. In clinical practice it proved necessary to resort either to indirect measurement of the effects of the circulating hormones (*e.g.*, the effect on eosinophils), or to determination of changes in the urinary excretion of these hormones as such, or in the form of one of their metabolic breakdown products.

The measurement of the changes in circulating eosinophils in response to ACTH is of value in the estimation of small amounts of circulating hormone only, since a maximal fall in circulating eosinophils is produced by relatively small quantities of 11,17-oxysteroids and hence further increase is not detectable.

For the measurement of a more marked increase in the output of adrenal cortical hormones the urinary excretion methods have been employed. The excretion of formaldehydogenic steroids parallels the excretion of 11,17-oxygenated steroids as determined chemically or by biologic assay, suggesting that part of this fraction represents actual adrenocortical hor-

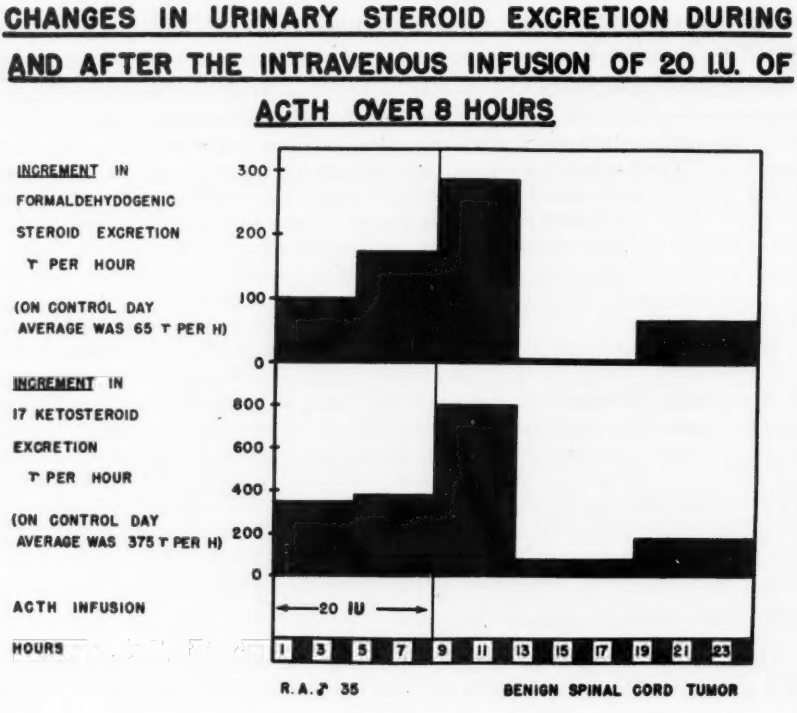

CHANGES IN URINARY STEROID EXCRETION DURING AND AFTER THE INTRAVENOUS INFUSION OF 20 I.U. OF ACTH OVER 8 HOURS
INCREMENT IN FORMALDEHYDOGENIC STEROID EXCRETION γ PER HOUR
(ON CONTROL DAY AVERAGE WAS 65 γ PER H)
300
200
100
0
INCREMENT IN 17 KETOSTEROID EXCRETION γ PER HOUR
(ON CONTROL DAY AVERAGE WAS 375 γ PER H)
800
600
400
200
0
ACTH INFUSION
20 IU
HOURS
1 3 5 7 9 11 13 15 17 19 21 23
R.A. ♂ 35
BENIGN SPINAL CORD TUMOR

FIGURE 1

mone. The value of this measurement, however, is limited by the *varying* amounts of such substances which are present in the conjugated form (normally 80 per cent or more) and thereby escape routine determination unless enzymic hydrolysis is also employed (19).

The urinary 17-ketosteroids represent hormones (or their breakdown products) of both adrenal and testicular origin, though their *increase* following adrenocortical stimulation with ACTH is of adrenal origin only. It is also apparent that at least a portion of the 17-ketosteroids appearing in the urine represent degradation products of other adrenocortical hormones and particularly of the 11,17-oxygenated steroids (20).

In view of these relationships it is not surprising that the urinary excretion of 17-ketosteroids and formaldehydogenic steroids during adrenocortical stimulation may be shown to follow parallel patterns. It may be seen in Figure 1 that during an eight-hour ACTH infusion both formaldehydogenic and 17-ketosteroid excretion begin to increase during the first four-hour period, reach a maximum during the four hours following the end of the infusion, and rapidly fall to normal during the next twelve hours.

Since the routine determination of urinary 17-ketosteroids is considerably less time-consuming than is the determination of formaldehydogenic steroids, and since obligatory hydrolysis eliminates the error resulting from varying degrees of conjugation, the determination of 17-ketosteroid excretion was selected as the most satisfactory method available for quantitating adrenocortical stimulation under the influence of ACTH. The changes in the response to ACTH following a two-stage bilateral adrenalectomy support the validity of this measurement in selected cases (Table 1).

TABLE 1. CHANGES IN THE INDICES OF ADRENAL CORTICAL RESPONSE ACCOMPANYING TWO-STAGE ADRENALECTOMY
(*J. M.*, 28, M., malignant hypertension)

Adrenal status	Eosinophil fall (per cent)*		17-KS excretion (mg. in 24 hrs.)	
	4 hours	10 hours	Control	Increase*
Preoperatively	—	100	15.1	+4.7
After unilateral adrenalectomy	92	98	10.0	+2.6
After bilateral adrenalectomy	21	6	4.9	+0.1

* During and after an eight-hour intravenous infusion of 20 I.U. of ACTH.

It is fully realized, however, that there are pathologic situations in which the metabolic degradation of 11,17-oxygenated steroids to 17-ketosteroids is impaired, or in which there is a more complete breakdown of

17-ketosteroids. In such instances, changes in 17-ketosteroid excretion cannot be directly related to differences in the level of activity of the adrenal cortices. An example of such a state may be found in cases of hepatic dysfunction (21).

Dose-response relationships

An essentially normal male subject was selected for these studies. A lower-limb paralysis, to which he was well adapted, prevented him from taking exercise and made him a relatively constant subject. When physiologic saline or 5 per cent dextrose in water was infused over an eight-hour period, no increase in the urinary excretion of 17-ketosteroids occurred during the corresponding 24-hour period. When increasing amounts of ACTH were added to the infusion, there was a proportional rise in the urinary 17-ketosteroid excretion up to an asymptotic level of a 14-mg. increase per twenty-four hours. This maximum was reached after ACTH had been increased to 15 or 20 I.U.; a further increase in the amount of ACTH above this critical dosage led to practically no further rise in 17-ketosteroid excretion (Fig. 2A).

In order to obtain reproducible experimental values, *at least* a three-day rest between individual eight-hour infusions must be allowed in order to assure the return to the initial state of adrenal cortical activity.

Even in a stable individual the value of the maximum 17-ketosteroid level reached will vary from time to time, presumably depending upon the basal functional state of the adrenal cortex or on differences in the over-all metabolic state of the individual (Fig. 2B). It is noteworthy that this maximum, though variable, appears to be reached at the same dosage level of ACTH in any given individual. When the increase in 17-ketosteroid excretion was expressed as a percentage of the maximum obtained within a week of the date of that particular experiment, and plotted against the appropriate dosage of ACTH, practically all the points obtained fell on or near a single rectangular hyperbole (Fig. 2C).

When the dose-response relationship was examined in different individuals, it was found that, although the type of resulting curve was identical, not only did the attained maximal value differ, but the ACTH dosage at which maximal stimulation occurred also varied. However, in all of the subjects tested, the dose of 20 I.U. was found to be well within the range of maximal effectiveness.

It may be concluded that in an essentially normal subject the immediate adrenal cortical stimulation obtained by the intravenous infusion of ACTH over a given time is a function of the ACTH dosage up to a critical amount over and above which no further adrenal cortical stimulation will be elicited. Under the conditions employed, *i.e.*, an eight-hour continuous in-

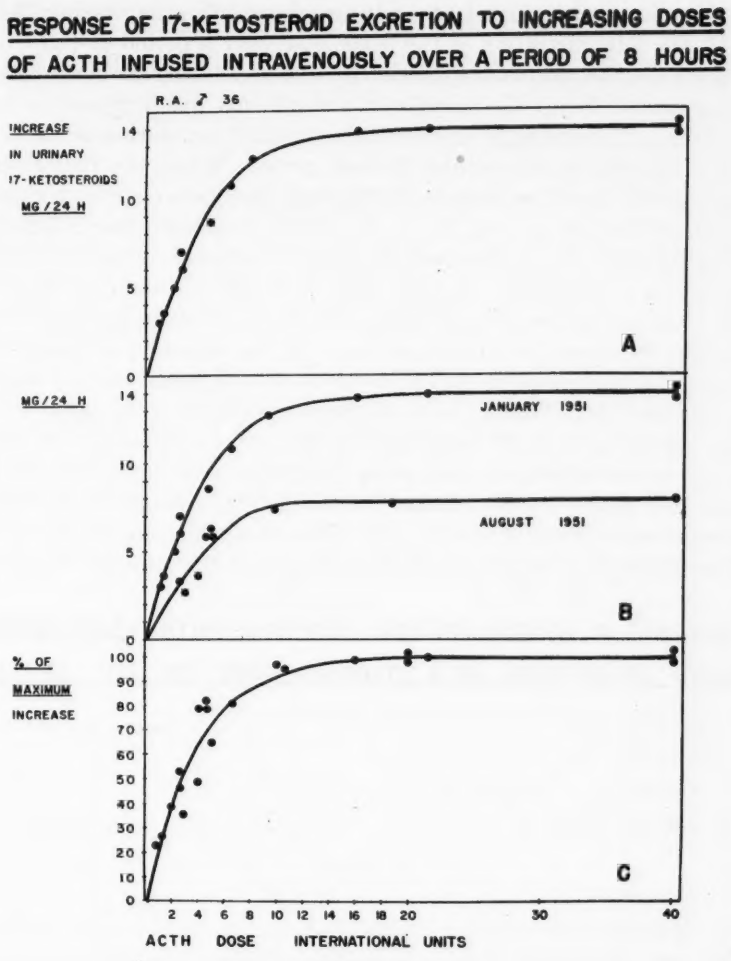


FIGURE 2

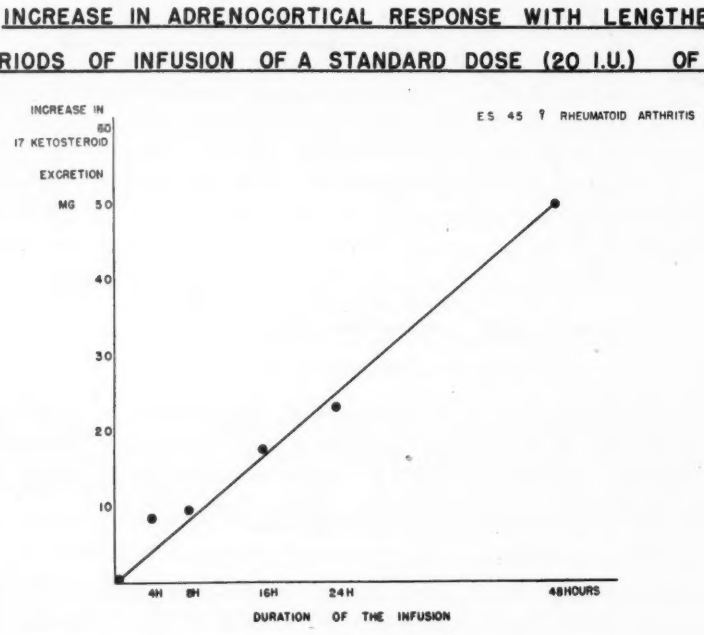
fusion, the maximum rise in 17-ketosteroids never failed to occur with 20 I.U. of ACTH. It is entirely possible that in chronically debilitated patients greater amounts of ACTH might be required to reach this maximum immediate activation of the adrenal cortex (22).

Time-response relationships

E.S., a patient with rheumatoid arthritis, on strict bed rest, was chosen for this study. The dosage selected was 20 I.U. of ACTH and this was kept constant throughout. When 20 I.U. of ACTH was infused over a 30-second

period, no increase in 17-ketosteroids was observed in the ensuing 24-hour period; the administration of the same dose over a period of eight hours increased the 17-ketosteroid excretion by 11.6 mg. during the corresponding 24-hour period. When the duration of the infusion was increased to forty-eight hours, 17-ketosteroid excretion rose by 22.2 mg. during the first, and by 27.6 mg. during the second 24-hour period. When the increment in 17-ketosteroid excretion obtained in this individual was charted against the duration of the infusion of 20 I.U. of ACTH, a straight-line relationship resulted (Fig. 3). It may be concluded that the adrenal cortical stimulation obtained by the infusion of a given dose of ACTH is directly proportional to the duration of the infusion within the time limits studied in this case.

Further evidence for the importance of the duration of intravenous infusion of a given dose of ACTH in terms of the adrenal cortical response was obtained by following the eosinopenia produced in the same patient. When injected over a 30-second period, 20 I.U. of lyophilized ACTH did not lead to any significant eosinopenia. The same material used in identical dosage over longer periods of time led to a fall in eosinophils, reaching its maximum approximately eight hours after the beginning of the infusion and maintained up to four hours after the end of the infusion. Thus, when



INCREASE IN ADRENOCORTICAL RESPONSE WITH LENGTHENING PERIODS OF INFUSION OF A STANDARD DOSE (20 I.U.) OF ACTH

FIGURE 3

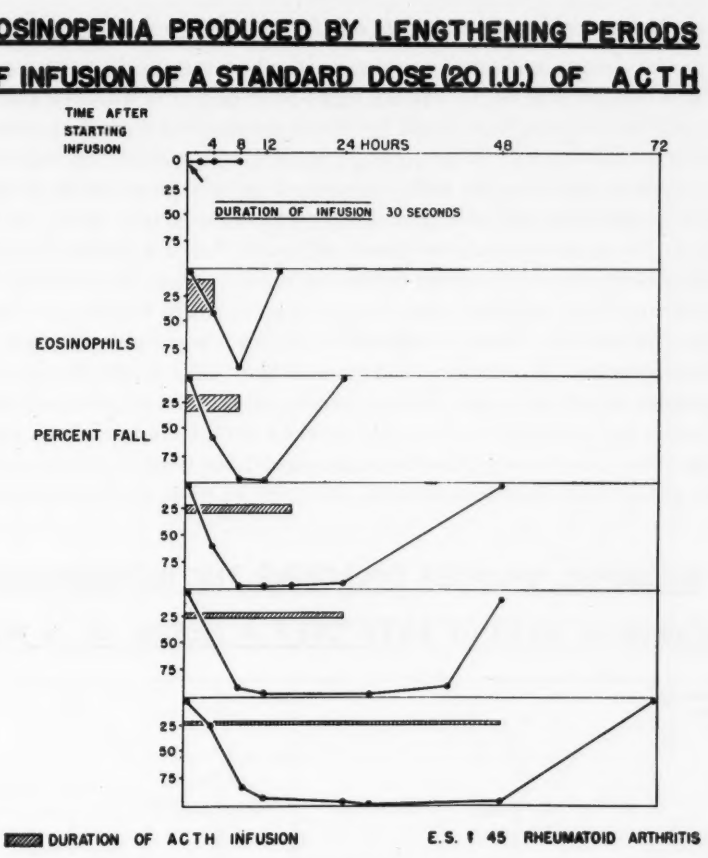


FIGURE 4

the 20-I.U. dose was given in a continuous infusion of forty-eight hours' duration, maximum eosinopenia was maintained for at least fifty-two hours. In all instances the eosinophil counts returned to normal within eight to twenty-four hours after the end of the infusion (Fig. 4).

The rather surprising finding of a total absence of eosinophil response following the injection of 20 I.U. of ACTH in thirty seconds in Patient E. S. led to an investigation of this point. It appears that in the particular, rather impure ACTH preparation used initially, a factor may have been present which annulled the eosinopenic effect of this preparation when administered as a rapid intravenous injection. Apparently such a factor was absent in some of the purer ACTH preparations which have since been used.

The eight-hour intravenous ACTH test for adrenal cortical competence

For the determination of adrenal cortical activation in response to the intravenous infusion of ACTH, an eight-hour period of administration was selected. This choice was made for the following reasons: A maximal fall in eosinophils occurs within eight to twelve hours after the beginning of the infusion and may be safely expected at ten hours. The eosinophils return toward normal at the end of twenty-four hours, rarely exceeding the pre-treatment level at that time. Although the 17-ketosteroid excretion is greatly increased during and for four hours following the period of ACTH infusion, no rebound depression occurs as a rule until twenty-four hours or more have elapsed. Thus an eight-hour period is a suitable time for noting adrenal cortical stimulation, in terms of the two criteria employed, *i.e.*, eosinophil depression and 17-ketosteroid rise. A twenty-four-hour urine collection will include any delayed rise in 17-ketosteroids without including the reactive depression. Above all, an eight-hour period of infusion represents a practical time interval from the point of view of the patient's comfort.

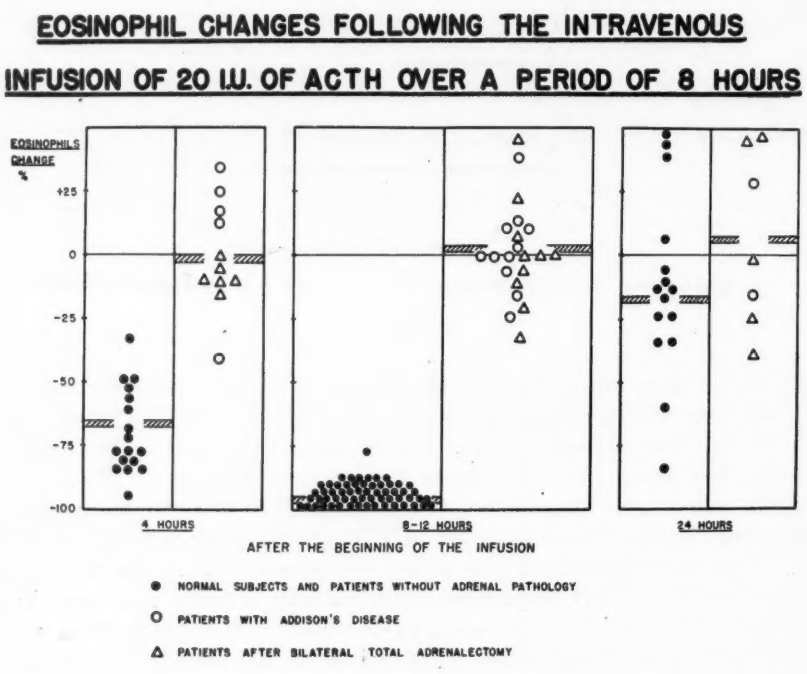


FIGURE 5.

(Note: 4-hour and 24-hour counts were made in a limited number of cases only.)

The eight-hour intravenous ACTH test has been applied in well over 100 subjects with or without adrenal pathology. Results are summarized in Figure 5 and Table 2.

The eosinophil fall at eight to twelve hours after the beginning of the infusion was found to be most reliable. In all of 66 normal subjects or patients without adrenal pathology there was a drop of 85 per cent or more in the level of circulating eosinophils. The wide margin between these results and those obtained in 11 patients with Addison's disease and in 10 patients following bilateral adrenalectomy is obvious and striking. Four patients showed a normal response to the intravenous infusion although they had previously failed to respond to intramuscular ACTH tests, thus indicating normal adrenal function. The failure to respond to intramuscular administration of ACTH must be attributed to local inactivation of the material in these particular patients all of whom had previously received therapeutic courses of ACTH (10).

The increase in 17-ketosteroid excretion during the day of the infusion varied from 1.8 to 17.6 mg. per twenty-four hours, with an average of 6.3 for the 41 tested normal subjects and patients without adrenal pathology.

TABLE 2. RISE IN URINARY 17-KETOSTEROID EXCRETION AND FALL IN CIRCULATING EOSINOPHILS FOLLOWING THE INTRAVENOUS INFUSION OF 20 I.U. OF ACTH OVER A PERIOD OF 8 HOURS

	No. of patients	Increment in 17-KS (mg./24 hrs.)	
		Mean	Range
Normal subjects and patients without evidence of adrenal pathology	41	6.3	1.8 to 17.6
Patients with Addison's disease	10	0.4	0 to 1.9
Hypertensive patients following bilateral total adrenalectomy	8	0.1	0 to 0.3
		8-12 hr. change in eosinophils (%)	
		Mean	Range
Normal subjects and patients without evidence of adrenal pathology	66	−97	−75 to −100
Patients with Addison's disease	11	+4	+40 to −25
Hypertensive patients following bilateral total adrenalectomy	10	+ 5	+80 to − 32

The patients with Addison's disease and those following bilateral adrenalectomy showed little or no change in their 17-ketosteroid excretion on the day of the infusion as compared to the control day (Table 2).

In pituitary hypofunction, repeated intravenous ACTH tests on three or four consecutive days were necessary to establish the diagnosis. Both the fall in eosinophils and the rise in 17-ketosteroid excretion, although poor on the first day in these patients, tended to show a gradual return to normal on the following days (Fig. 6). In contrast, in 8 patients with pri-

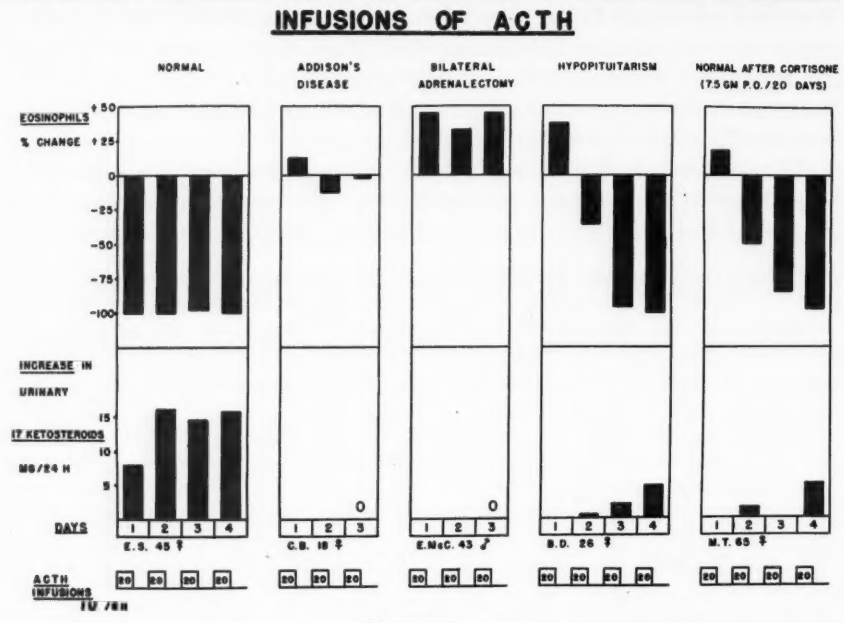


FIGURE 6

mary adrenal cortical insufficiency, the responses to repeated eight-hour tests remained abnormal throughout.

Following cortisone therapy, patients tend to show a pattern similar to that found in hypopituitarism. This is illustrated by Patient M. T., a 65-year-old diabetic female, with generalized exfoliative dermatitis, who was given 7,500 mg. of cortisone *orally* over a period of twenty days. Intravenous ACTH infusions were given on two consecutive days prior to, and on the second to fifth day after cortisone therapy. The eosinophil responses obtained are shown in Figure 7. It is apparent that on the second day after cessation of oral cortisone no adrenal cortical activation was ob-

tained, and it was not until the fourth day that normal eosinophil response was reached. Since it has been shown that 20 I.U. of ACTH intravenously over a period of eight hours represents essentially a maximal stimulation for a normal adrenal cortex, it must be assumed that, functionally, on the second day after the cessation of cortisone this patient showed frank adrenocortical insufficiency. On this day the immediate adrenocortical reserve was practically zero, even though the potential adrenal cortical

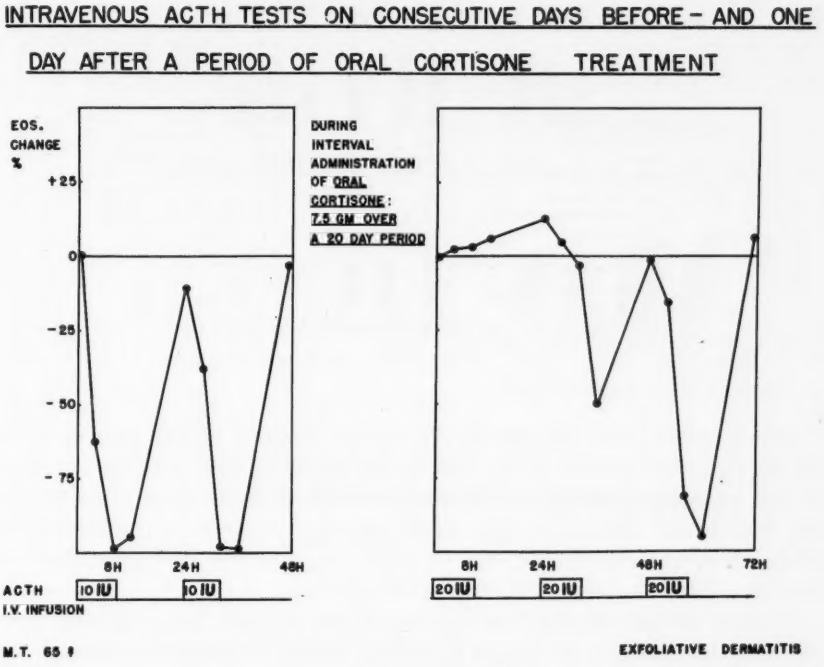


FIGURE 7

reserve was probably not impaired. When intramuscular ACTH administration was continued during oral cortisone therapy, adrenal activation followed administration of exogenous ACTH, although presumably anterior pituitary inhibition was present.

Repeated daily ACTH infusions

Twenty patients were studied (Table 3). The number of consecutive eight-hour daily ACTH infusions varied from five to thirty. In 3 patients two courses, and in 1 patient three courses of infusions were given over a period of six months.

TABLE 3. 17-KETOSTEROID EXCRETION DURING REPEATED DAILY ACTH INFUSIONS

Patient	Sex	Diagnosis	Total no. of consecutive daily infusions (8 hours)	No. of courses	17-KS excretion (mg./24 hrs.)			Group*
					Control	First infusion	Maximal	
R. A.	M	Benign spinal cord tumor	14	1	16.5	31.8	9.8	1
G. R.	M	Psoriasis	10	2	8.4	19.0	25.0	1
A. L.	M	Gout	8	1	9.0	13.4	23.3	1
E. S.	F	Rheumatoid arthritis	5	2	7.3	14.9	23.6	1
V. F.	F	Multiple sclerosis	21	1	8.4	13.3	22.4	1
P. L.	F	Anasarca	7	1	4.0	7.6	20.0	1
P. McD.	M	Rheumatoid arthritis	16	3	9.6	15.5	23.5	1
R. F.	M	Myeloid metaplasia	16	1	15.6	—	25.1	1
S. L.	F	Psoriasis	9	1	6.0	—	20.8	1
G. R.	M	Rheumatic fever	20	1	16.2	23.0	37.0	2
M. J.	M	Boeck's sarcoid	23	2	2.3	7.8	21.3	2
R. K.	M	Psoriasis	12	1	5.3	7.1	22.7	2
H. M.	F	Multiple sclerosis	28	1	3.2	11.8	34.8	3
A. J.	F	Multiple sclerosis	30	1	7.3	—	33.9	3
G. J.	F	Lupus erythematosus disseminatus	16	1	2.2	—	25.1	3
M. McN.	F	Diabetes; psoriasis	12	1	8.8	12.7	15.8	4
G. S.	F	Diabetes; arthritis	18	1	1.0	5.3	9.4	4
M. A.	F	Lupus erythematosus disseminatus	17	1	5.1	8.0	12.1	4
B. S.	F	Leukemia	12	1	5.1	9.7	11.3	4
J. L.	F	Rheumatic heart disease	12	1	1.4	—	4.8	4

* According to classification described in text.

In all patients the *circulating eosinophils* dropped 85 per cent or more on the first day and usually to zero on the following days. Counts obtained in the morning, however, before starting the infusion, were often within the individual's normal range. There was no decrease in the eosinophil response either during the five to thirty days of the first treatment or during subsequent repeated courses of infusions.

The *17-ketosteroid excretion* showed a rise on the first day and then continued to increase for two or more days before leveling off. Although the responses obtained all followed essentially the same pattern, four general groups could be distinguished (Table 3). Representative cases are shown in Figure 8.

Group 1, in which a maximal 17-ketosteroid output was rapidly obtained and then maintained, is represented by R. A. (Fig. 8). The 17-ketosteroid excretion rose to a maximum within three days, and was then essentially maintained without further rise until the end of the period of infusions. Another patient in this group was treated for twenty-one days, with similar results; moreover, increasing the dosage of ACTH did not lead to any further rise in 17-ketosteroid excretion.

Group 2, showing a two-step rise in 17-ketosteroids, may be illustrated by the following cases: G. R., a 26-year-old male (Fig. 8) was admitted

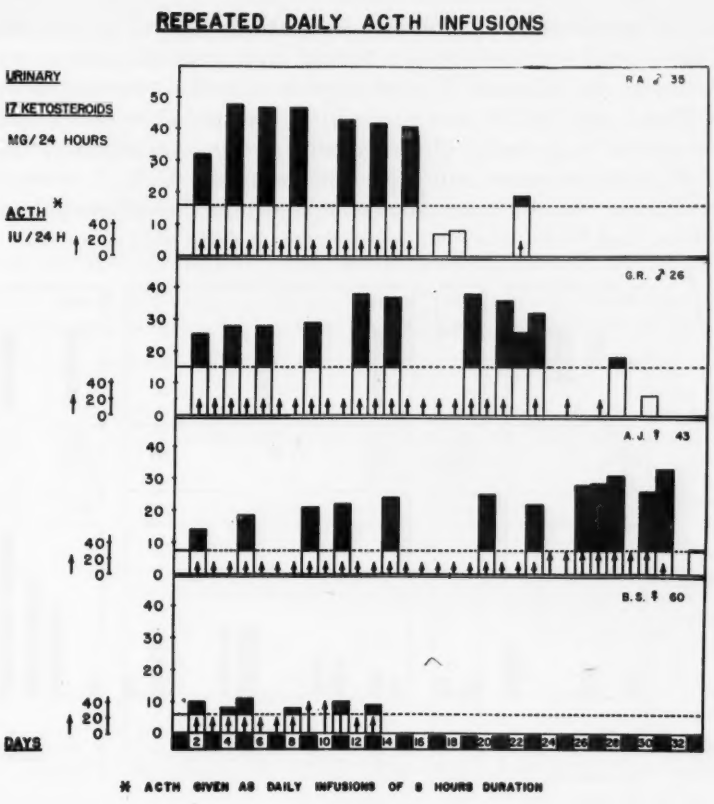


FIGURE 8

with acute rheumatic fever. Within three days of instituting therapy he was asymptomatic except for some persistence of effusion in the left knee and in both ankles. Within nine days from the start of therapy this last symptom had disappeared and he remained completely asymptomatic thereafter. The 17-ketosteroid excretion showed a definite two-step increase, reaching an initial level on the third day, and a second and final level on the eleventh day. M. J., a 19-year-old colored male with generalized Boeck's sarcoid (Fig. 9) had been admitted primarily because of steadily decreasing pulmonary reserve and dyspnea. He proved to be resistant to a second course of ACTH intramuscularly. He was given twenty-three eight-hour intravenous infusions, resulting in almost complete clearing of the diffuse pulmonary infiltration within ten days. A marked two-step response occurred, 17-ketosteroid excretion reaching the first level on the fifth, and the second level on the fourteenth day. R. K., a 50-year-old male with

generalized psoriasis and exfoliative dermatitis, reached a first plateau of 17-ketosteroid excretion on the second, and a second plateau on the eighth day of the infusions. It is of interest that all 3 patients had widespread disease and that the second rise in 17-ketosteroid excretion occurred at a time when final control of the extensive lesions was obtained—E. R., when all joint symptoms and fever had disappeared, M. J. when there was no further improvement shown by x-ray in the diffuse pulmonary sarcoidosis, and R. K. after complete clearing of the skin.

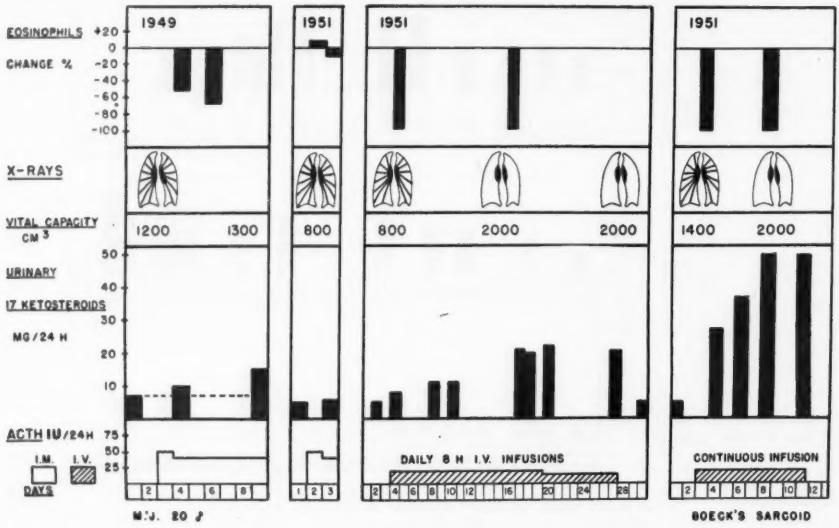


FIG. 9. Adrenocortical response of a patient with generalized Boeck's sarcoid to ACTH given intramuscularly, in daily intravenous infusions, and in a continuous intravenous infusion.

The response in Group 3 (A. J., Fig. 8) is characterized by a gradual and progressive increase in 17-ketosteroid excretion without the attainment of a definite maximum, during periods of treatment lasting up to thirty days.

The fourth group (B. S., Fig. 8) differed from the others in that the 17-ketosteroid values reached were definitely lower (8–15 mg. per twenty-four hours, as opposed to 20–50 mg.) than in the three other groups.

In conclusion, we may say that in all patients studied with daily infusions of ACTH (from five to thirty), the 17-ketosteroid excretion increased for the first few days, tending toward a variable maximum which was maintained up to the end of the infusions. In this study no significant decrease in 17-ketosteroid excretion occurred while daily maximal ACTH

stimulation was carried out for eight hours and no clinical escape was noted. The termination of the series of infusions, however, led to a definite, though transient, rebound in eosinophils, 17-ketosteroid excretion and occasionally in symptomatology. In addition, there was a poorer 17-ketosteroid response to an eight-hour ACTH infusion, when the values obtained two to three days after treatment were compared to those in the period before starting the infusions (R. A., Fig. 8).

Continuous intravenous ACTH administration

The effects of this form of adrenal cortical stimulation have been studied in 7 patients who were given ACTH intravenously as a continuous infusion during periods of from three to fifteen days (Table 4). The continuous intravenous administration of ACTH was found to be the most effective form of adrenocortical stimulation and the activation obtained was greater than that following twenty or more days of daily intravenous infusions of eight hours' duration. This comparison was carried out in the previously mentioned 19-year-old male with generalized sarcoidosis (M. J., Fig. 9). During his first admission in 1951 he was given twenty-three daily eight-hour infusions of ACTH (20 I.U.) with good clinical results. Within *ten days* the diffuse pulmonary infiltration had almost completely disappeared when viewed by x-ray and the vital capacity had increased from 800 to 2,000 cc. The adrenal activation obtained was good, despite a very low initial 17-ketosteroid excretion. The maximal 17-ketosteroid excretion obtained was 21.3 mg. per twenty-four hours. Within two months after discharge he showed a slow partial relapse and on his second admission was given a continuous ACTH infusion (21 I.U. daily) of ten days' duration. Within *five days* the pulmonary infiltration had again markedly

TABLE 4. 17-KETOSTEROID EXCRETION DURING CONTINUOUS ACTH INFUSIONS

Patient	Sex	Diagnosis	Days of continuous ACTH infusion	17-KS excretion (mg./24 hrs.)	
				Control	Maximal
E. J.	F	Erythema nodosum	8	2.9	32.0
D. A.	F	Phlebitis	10	5.4	37.1
M. J.	F	Lupus erythematosus disseminatus	4	2.1	34.2
R. A.	M	Benign spinal cord tumor	3	13.1	43.4
M. J.	M	Boeck's sarcoid	10	4.3	61.8
G. R.	M	Psoriasis	3	8.4	38.3
C. C.	F	Myeloma	15	3.9	23.2

improved and the vital capacity had returned to 2,000 cc. On this occasion 17-ketosteroid excretion rose from 4.3 mg. per twenty-four hours to 57 mg. on the sixth day and did not increase any further during the last four days of the infusion, even though the ACTH dosage was tripled on the last day. A single formaldehydogenic steroid determination on the last day of the continuous ACTH infusion gave a value of 44 mg. per twenty-four hours, the highest value obtained in this laboratory.

An interesting comparison between the effectiveness of ACTH given intramuscularly and by the continuous intravenous route of administration

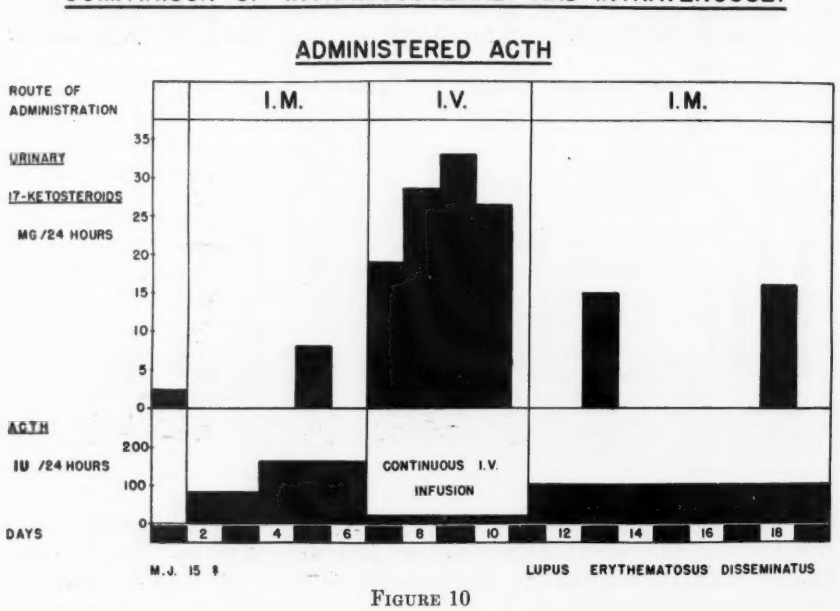


FIGURE 10

is shown in Figure 10. As judged by 17-ketosteroid excretion, the adrenal cortical stimulation obtained from a continuous intravenous drip of 20 I.U. of ACTH daily was double or more the stimulation produced by 160 I.U. of the hormone given intramuscularly in four divided doses. It is also apparent that after stimulation of the adrenal cortex by the intravenous route, intramuscular doses of ACTH similar to those previously employed were considerably more effective.

The minimal dose required for the maintenance of maximal adrenal cortical stimulation under continuous ACTH administration in one individual was sought in E. d'A., a 19-year-old girl with recurrent erythema nodosum, who was given a total of 30 I.U. of ACTH daily for

four days, then 20 I.U. daily for three days, and finally 7.5 I.U. daily for one day (Fig. 11). From a control value of 2.9 mg. per twenty-four hours, the urinary 17-ketosteroid excretion rose steadily during the 30-I.U. ACTH dosage, continued to rise in spite of a reduction in ACTH dosage to 20 I.U. daily, reached a maximum value of 32 mg. per twenty-four hours, and then fell sharply during an ACTH dosage of 7.5 I.U. Thus it appeared that the minimal daily ACTH dose necessary for maintenance of maximal stimulation lay somewhere between 7.5 and 20 I.U. for this patient.

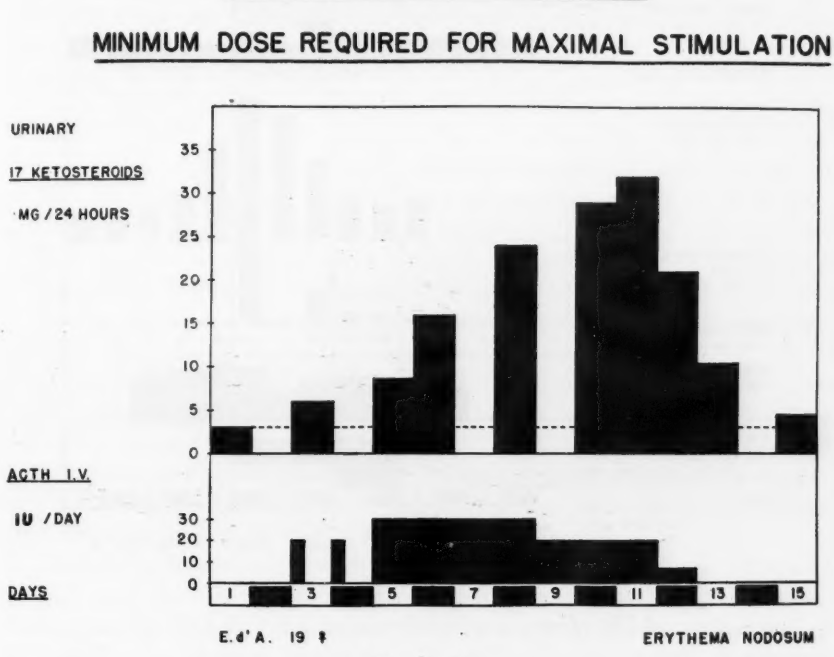


FIGURE 11

Finally, the over-all metabolic effectiveness of continuous intravenous ACTH administration was demonstrated in R. A., a 35-year-old male. After five days on a constant diet (2,090 calories, 210 Gm. carbohydrate, 72 Gm. protein, 83 Gm. fat, 73 mEq. sodium, 92 mEq. potassium and 2,500 ml. total fluid) a continuous infusion of 5 per cent dextrose in water was started, 1,500 ml. being given per day. After two control days, a dose of 30 I.U. of ACTH per day was added to his dextrose infusion and this was maintained for three days, all infusions being discontinued thereafter. The results are shown in Figure 12.

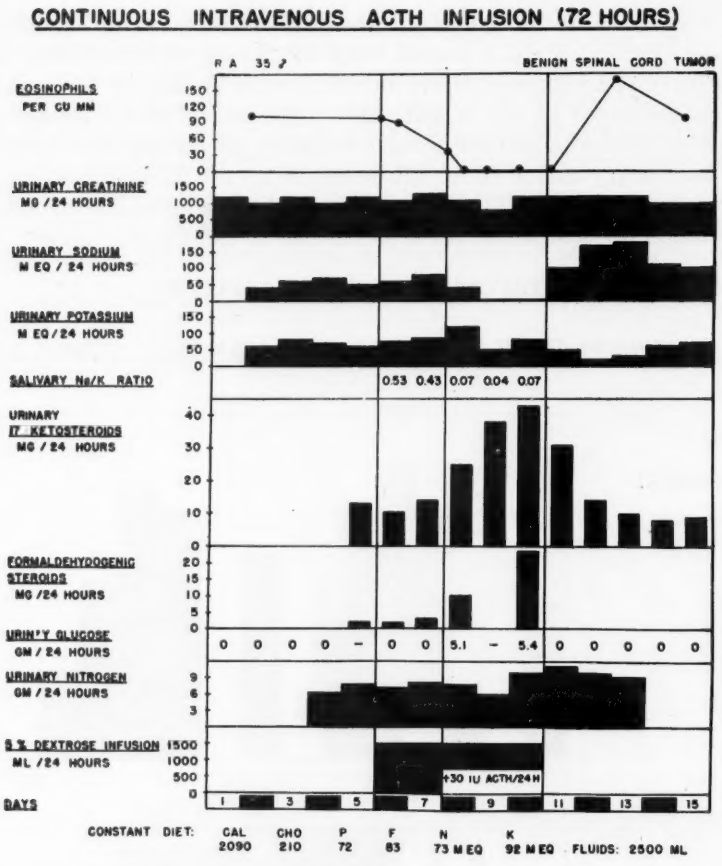

CONTINUOUS INTRAVENOUS ACTH INFUSION (72 HOURS)
R A 35 ♂
BENIGN SPINAL CORD TUMOR
EOSINOPHILS PER CU MM
URINARY CREATININE MG / 24 HOURS
URINARY SODIUM M EQ / 24 HOURS
URINARY POTASSIUM M EQ / 24 HOURS
SALIVARY Na/K RATIO
0.53 0.43 0.07 0.04 0.07
URINARY 17 KETOSTEROIDS MG / 24 HOURS
FORMALDEHYDOGENIC STEROIDS MG / 24 HOURS
URIN'Y GLUCOSE GM / 24 HOURS
0 0 0 0 – 0 0 5.1 – 5.4 0 0 0 0 0
URINARY NITROGEN GM / 24 HOURS
5 % DEXTROSE INFUSION ML / 24 HOURS
+30 I.U. ACTH/24 H
DAYS
CONSTANT DIET: CAL 2090 CHO 210 P 72 F 83 N 73 M EQ K 92 M EQ FLUIDS: 2500 ML

FIGURE 12

The major ACTH effects previously described (13, 14) were apparent and unusually pronounced. This is particularly true of the effects on electrolytes. During the combined second and third days of ACTH administration more than 5 liters of the patient's urine contained a total of of only 3 mEq. of sodium. Potassium diuresis was evident only on the first day; the salivary Na/K ratio (23) dropped from 0.53 and 0.43 while on infusions of dextrose alone, to 0.07 and 0.04. During ACTH infusion, 17-ketosteroid and formaldehydogenic steroid excretion increased markedly and in parallel fashion. There was but a mild transient glycosuria. Subjectively, the patient felt restless and tense, but there were no other remarkable clinical features.

If the stimulation obtained was well marked, so was the "rebound"

which followed. On the second and third day after the end of the infusion, particularly, the patient felt extremely weak and tired. On these two days the urinary excretion of sodium increased to 189 and 194 mEq., respectively, which represents three times the dietary intake of sodium on these days and exceeds the amount retained previously. There was definite potassium retention, an unusually high eosinophil count and a fall below normal of the 17-ketosteroid excretion by the third and fourth days, with a subsequent return to normal.

There were minor changes in blood chemistry values, in the same direction as those described following repeated intramuscular ACTH injection (13, 14). Only one unusually marked change was noted; the cholesterol level fell during ACTH infusion from control values of 240 and 180 mg., to 75 mg. per 100 ml. of serum.

ACTH resistance

A number of patients receiving repeated courses of certain types of ACTH (Armour) have shown increasing unresponsiveness to the hormone, when given by intramuscular injection. Occasionally a complete lack of response ensued. In such patients dosages as high as 100 mg. of ACTH daily, in four divided doses, have produced no evidence of adrenocortical stimulation. This phenomenon has been termed ACTH resistance (10).

Of approximately 100 patients treated with prolonged (more than two weeks) and repeated (two or more) courses of lyophilized Armour hog ACTH, 8 patients showed frank resistance to the hormone administered *intramuscularly* and could be studied in that respect. It was of particular interest to note that all of these patients showed an entirely normal response to the same type of ACTH given *intravenously.*

Adrenocortical stimulation produced by an intramuscular injection of ACTH appears to be dependent upon the following factors: 1) the adrenocortical reserve of the individual tested; 2) the speed of local absorption of ACTH; 3) the total amount of ACTH absorbed and therefore the degree of local inactivation, whether by simple tissue fixation or actual destruction; and 4) the possible presence of circulating antihormones or antibodies.

In the group studied, the adrenocortical reserve of the test subject was demonstrated by a normal response to intravenous ACTH. An attempt was made to minimize the differences in the speed of local absorption by comparing intramuscular and intravenous *infusions* of identical doses of ACTH, infused over the same period of time. Thus, local tissue fixation or inactivation remained as the chief variable in determining the effectiveness of the intramuscularly, as compared with the intravenously, adminis-

TABLE 5. COMPARATIVE RISE IN URINARY 17-KETOSTEROIDS FOLLOWING AN 8-HOUR INFUSION OF ACTH INTRAVENOUSLY AND INTRAMUSCULARLY

Subject	Dose and type of ACTH	Rise (mg.) in urinary 17-KS following	
		Intravenous ACTH	Intramuscular ACTH
B. G., F, 58	20 I.U. ACTHAR, Armour J-16609R*	5.9	2.3
L. B., F, 26	20 I.U. ACTHAR, Armour J-16609R	9.3	4.5
D. H., M, 24	10 I.U. ACTH, Parke, Davis X-4865**	8.7	13.5
E. W., F, 17	10 I.U. ACTH, Parke, Davis X-4865	5.1	4.8

* A lyophilized preparation obtained from hog pituitaries, containing significant amounts of contaminating protein.

** A purified and practically protein-free preparation.

tered hormone. The intravenous and the intramuscular effects of identical doses of ACTH on 17-ketosteroid excretion were compared in this fashion in subjects who had *not previously received ACTH* (Table 5). A preparation of relatively small molecular size prepared by glacial acetic acid extraction and purification by absorption-elution on cellulose was of equal potency in both instances, whereas material prepared by isoelectric precipitation of the mildly acid pituitary extract and containing considerably more protein per unit of activity, proved significantly less potent when given intramuscularly. It would therefore appear that greater fixation, more inactivation, or both, were present with the latter preparation when given intramuscularly.

This tendency of certain types of ACTH to provoke a decrease in the response after intramuscular administration, as compared to the response obtained intravenously, is markedly exaggerated in patients *having previously received therapeutic doses of ACTH*.

E. S., a patient with induced ACTH resistance following five previous courses of intramuscular ACTH treatment, showed no eosinophil or 17-

ketosteroid response to ACTH given intramuscularly, either as a forty-eight-hour test or as an eight-hour intramuscular infusion of 20 I.U. Yet, 20 I.U. of ACTH given intravenously over an eight-hour period produced a 98 per cent fall in eosinophils and a 9.0-mg. rise in 17-ketosteroid excretion, a response well within the normal range (Fig. 13). In 1949 this pa-

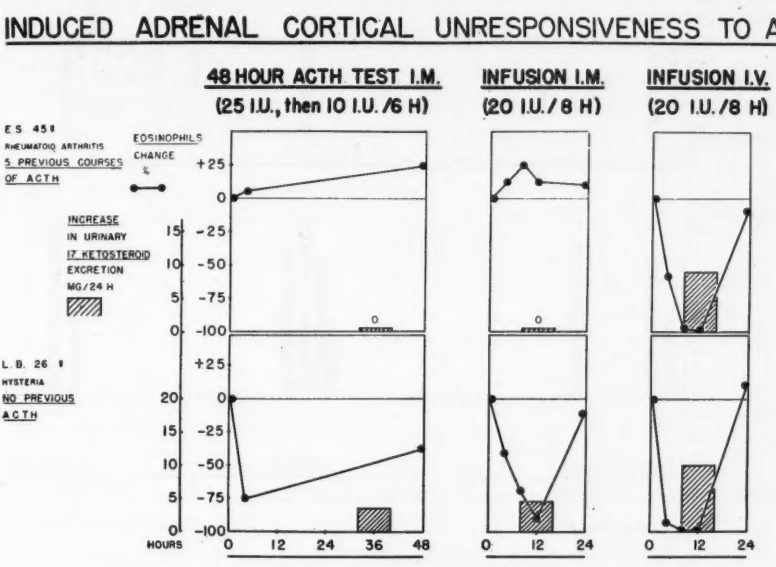


FIGURE 13

tient had responded satisfactorily to a forty-eight-hour intramuscular ACTH test with a 60 and a 92 per cent fall in eosinophils at four and at forty-eight hours, respectively, and a 6.0-mg. rise in 17-ketosteroid excretion. Thus, in this patient most of the ACTH injected intramuscularly was apparently locally inactivated. For comparison, Patient L. B. (not resistant to ACTH) is included (Fig. 13) as an example of normal response.

Patient M. J. (Fig. 9) illustrates the same point. He responded to intramuscular ACTH in 1949 but not in 1951, yet showed an excellent response to intravenous ACTH. Patient P. McD., a 39-year-old taxi driver with rheumatoid arthritis, was given three courses of intramuscular and intravenous ACTH therapy between February and June, 1951 (Fig. 14). There was no change in the response to intravenous ACTH infusion during that time; but whereas in March a fall in eosinophils, a rise in 17-ketosteroid excretion and symptomatic improvement occurred on ACTH given intramuscularly, in May even larger doses of intramuscular ACTH failed to control symptoms or to lead to a significant fall in circulating eosinophils.

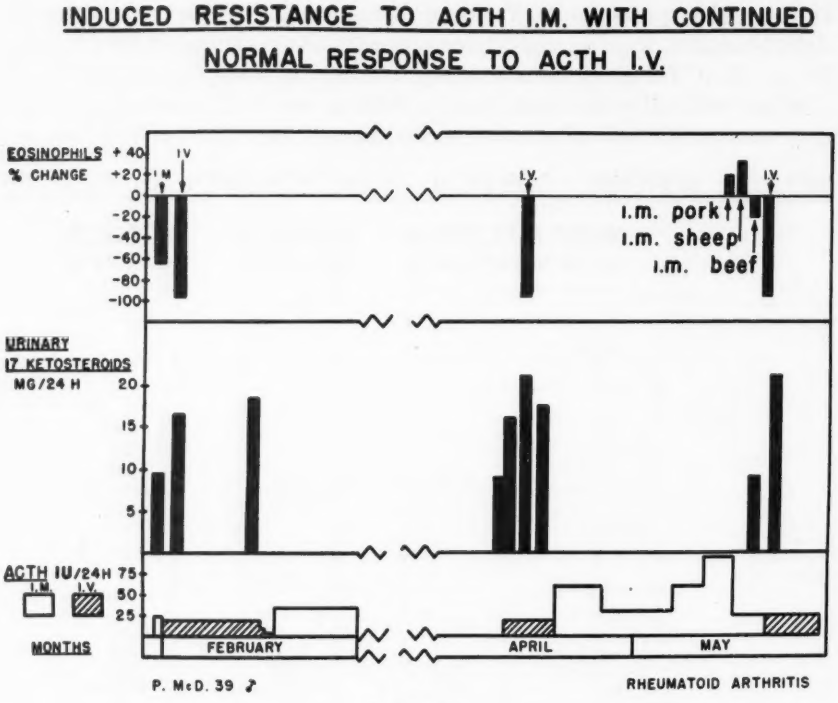


FIGURE 14

Is the tissue inactivation of certain types of ACTH in patients with actual ACTH resistance influenced by the nature of the contaminating protein? In some cases studied, changing the animal species from which ACTH was obtained reinstituted a more nearly normal response to intramuscular ACTH; in others, however, complete resistance to ACTH preparations obtained from pork, beef and sheep pituitaries was found (Fig. 14). It would appear that the nature of the contaminating material may in certain cases influence induced ACTH resistance (24).

The experiments outlined emphasize the importance of tissue inactivation as opposed to intravascular inactivation, when ACTH resistance is present. Inasmuch as one might assume a slow diffusion of the ACTH from the site of injection into the blood stream, the effect of ACTH should be practically identical, whether given as an intramuscular or as an intravenous infusion. Nevertheless a marked loss of potency ensues only when the intramuscular route is used. The existence of a good response by the intravenous route indicates that circulating antibodies to ACTH or its accompanying nonspecific proteins do not play an important role in ACTH

resistance of the type described, even though such antibodies have been demonstrated by others in animals (25) and in man (24, 26). In well over eight hundred intravenous infusions, unresponsiveness due to antibody formation has not been observed.

The biologic estimation of ACTH activity

As has already been pointed out, the intravenous infusion of small amounts of ACTH over a period of eight hours leads to all the major known metabolic changes attributed to ACTH, within a single twenty-four-hour period. This offers obvious advantages for the full metabolic trial and assay *in man* of material of unknown potency, particularly when only minimal amounts are available.

As an example, the study of two such substances may be presented: 1) The high-potency Fraction 12 obtained from standard ACTH, fractionated by pH adjustment, peptic hydrolysis and counter-current distribution of the trichloroacetic acid-soluble fraction, as prepared by Lesh *et al.* (12). The material was made up in propylene glycol solution, 1 ml. of which contained 0.4 mg. of solid, of which 0.34 mg. was organic material. 2) A partly purified corticotropin preparation obtained by Astwood and collaborators (Fraction C) made up in dilute hydrochloric acid and containing 0.4 mg. of solid per ml. (11).

A 20-year-old student was given a constant diet consisting of 400 Gm. of carbohydrate, 150 Gm. of protein, 240 Gm. of fat, 200 mEq. of sodium, 115 mEq. of potassium and 2,000 ml. of fluid per day. After five days on the diet he was given 10 I.U. of Armour ACTH in 500 ml. of 0.72 Gm. per cent salt solution over eight hours, followed at intervals of three days by 0.4 mg. of the "Lesh" and 0.3 mg. of the "Astwood" preparations. After a further interval of four days, 500 ml. of salt solution alone was infused as a control. On all occasions the amount of fluid, as well as the salt infused, was subtracted from the dietary intake of the corresponding twelve-hour period. Urine collections were made over twelve-hour periods and blood samples were drawn at the appropriate times.

The results of the metabolic experiment represented in Figure 15 were of a comparable magnitude with both the standard and the two purified ACTH preparations, although they were somewhat more pronounced with the material of Lesh. Both purified ACTH preparations appeared to be at least 20 to 25 times as active per unit of weight as the Armour Standard LA-1-A.

From this type of experiment it was concluded that both of these highly purified ACTH preparations, though obtained by entirely different processes of purification and from different starting materials, produced metabolic changes essentially identical with those obtained with the standard

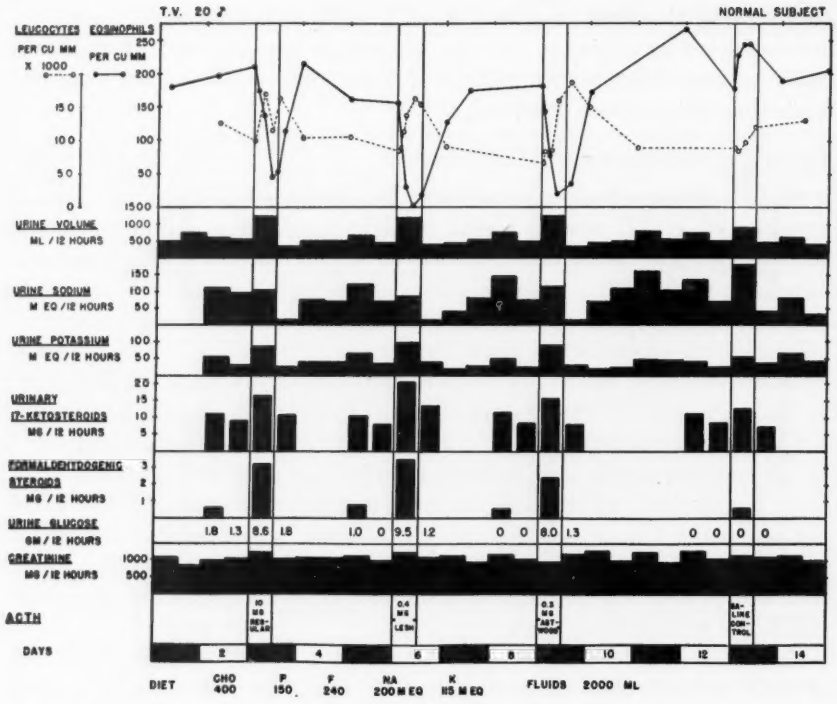

T.V. 20 ♪
NORMAL SUBJECT
LEUCOCYTES PER CU MM X 1000
EOSINOPHILS PER CU MM
URINE VOLUME ML / 12 HOURS
URINE SODIUM M EQ / 12 HOURS
URINE POTASSIUM M EQ / 12 HOURS
URINARY 17-KETOSTEROIDS MG / 12 HOURS
FORMALDEHYDOGENIC STEROIDS MG / 12 HOURS
URINE GLUCOSE GM / 12 HOURS
1.8 1.3 8.6 1.8 1.0 0 9.5 1.2 0 0 8.0 1.3 0 0 0 0
CREATININE MG / 12 HOURS
ACTH
10 MG REGULAR
0.4 MG LENTE
0.2 MG "CRYSTALLINE" ACTH-WOOD
SALINE CONTROL
DAYS
2 4 6 8 10 12 14
DIET CHO 400 P 150 F 240 NA 200 M EQ K 115 M EQ FLUIDS 2000 ML

FIG. 15. Metabolic activity of two highly purified small molecular ACTH preparations as compared to the Armour Standard LA-1-A.

ACTH preparations in man. This suggests a close similarity of the as yet unidentified "active principle" in these different preparations.

The eight-hour intravenous infusion of ACTH has also been used to attempt *the biologic assay of ACTH in man.* As previously described, a constant relationship has been demonstrated between the dosage of intravenous ACTH and the increase in 17-ketosteroid excretion expressed as the percentage of the maximum rise for any given individual (Fig. 2C). This relationship takes the form of a rectangular hyperbole. By using the logarithm of the dose in expressing this relationship, one obtains a sigmoid curve with a linear portion at the center. Such a curve, derived from that shown in Figure 2C, is shown in Figure 16A.

In order to determine the potency of an unknown ACTH preparation, one must administer the material at two distinct dosage levels, one sufficiently small (2–5 I.U.) to fall on the steep and linear portion of the sigmoid curve, and the other well within the range of maximal stimulation (20 I.U. or over), so as to compute the rise in 17-ketosteroid excretion in

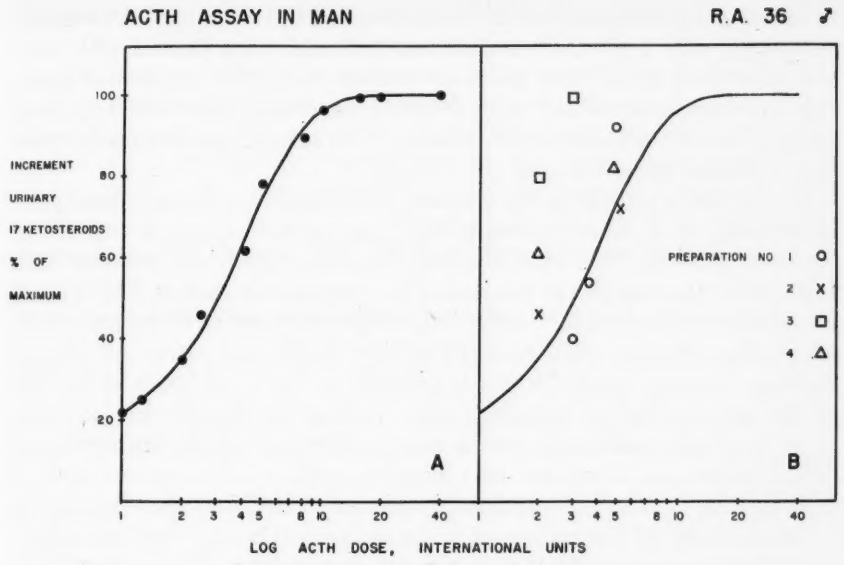


FIGURE 16

terms of percentage of the maximum. When this value is transposed onto the calibration curve worked out for the same individual, the logarithm of the equivalent dose, the dose, and hence the activity of the preparation may be obtained. In Figure 16B the assay of a number of commercial preparations in Subject R. A. is graphically represented. The paucity of our data does not allow for a statistical evaluation of the results obtained and represents only a rough approximation. One may, however, conclude that Preparation 3 is well above stated potency, and the others close to it. Such a procedure might be useful as a practical assay method in man, if a sufficiently large number of determinations were carried out for any given preparation.

Clinical use of intravenous ACTH

Well over 800 ACTH infusions have been given to approximately 200 patients and normal subjects. This group included a number of patients with marked allergic background and some patients who showed transient local induration and pain at the site of intramuscular ACTH injections.

A frequent observation was the early appearance of a generalized flush of the skin, sometimes accompanied by a subjective impression of warmth and often by sweating. It was thought that this was most probably due to physiologic activity of the drug and did not represent an allergic phenomenon.

In the group including normal subjects and all patients without adrenal pathology, only 1 allergic reaction was observed—in a patient with gout who had been given four previous courses of ACTH intramuscularly, and in whom generalized mild urticaria developed approximately four hours after the beginning of the infusion. This cleared spontaneously while the procedure was continued.

In the group including the patients with Addison's disease, panhypopituitarism, and those following bilateral adrenalectomy, 2 out of 18 patients who received single infusions showed a mild febrile reaction four hours after the end of the infusion. The temperature rose to 102° F. and returned to normal within twelve hours. However, out of 12 patients with adrenal insufficiency who received two or more consecutive ACTH infusions, 4 showed a definite febrile reaction within a few hours of the end of the second, third or fourth infusion. During this febrile reaction 1 patient went into mild crisis, which was rapidly controlled with cortisone. Blood cultures in 2 patients gave negative results. All of these reactions followed the use of the lyophilized type of ACTH. They were thought to be most likely of the nature of a simple anaphylactoid reaction. Their total absence in individuals with normal adrenal function was thought to be related to the high levels of circulating adrenal steroids during and after ACTH infusions, which might well be sufficient to suppress the manifestations of any such allergic reaction. It is apparent that in patients with adrenal insufficiency care should be exercised when using intravenous ACTH.

The complications of ACTH therapy are inherent in its physiologic activity. These complications, as well as measures useful for their prevention, have been adequately described elsewhere (27, 28, 29). No major differences were observed when using the intravenous route of administration. However, since the adrenal cortical stimulation produced was particularly effective, it appears logical that complications developed somewhat more rapidly and were more pronounced. This was true in particular of the rounding of the face, of the electrolyte imbalances, and of the severity of hypoadrenal symptoms in the rebound phase. All patients receiving intravenous ACTH therapy for more than two days were, therefore, routinely given the hospital low-salt diet (approximately 2 Gm. of NaCl daily) and, in the absence of renal pathology, they were given from 1.8 to 5.4 Gm. of potassium chloride daily as enteric coated tablets. When treating patients with border-line congestive heart failure, a strict "salt-free" diet was used. Serum levels of sodium and potassium were checked when indicated.

Whenever feasible, abrupt discontinuation of the hormonal therapy was avoided, realizing that the adrenal becomes hypoactive within one to two

days after discontinuing intravenous ACTH (Figs. 9 and 12). A single eight-hour infusion on the second and fifth days after the end of prolonged periods of daily or continuous infusion was usually adequate to prevent too precipitous a fall in adrenal hormone production at that time. When these precautions were observed, no such complications were noted.

The group of patients receiving continuous intravenous infusions for periods of three days or more represent a special problem. Their physiologic response was of such intensity and changes occurred so abruptly, both during and after therapy, that unusually close observation was necessary.

TABLE 6. REACTIVATION WITH INTRAVENOUS ACTH OF ADRENAL CORTEX SUPPRESSED BY ORAL CORTISONE

Patient	Previous cortisone		Daily infusions of 20 I.U. ACTH over eight hours								No. of infusions required for adequate response
			Eosinophil fall, per cent				17-KS increase, mg./24 hr.				
			1	2	3	4	1	2	3	4	
	Daily dose (mg.)	Days	Days				Days				
L. T.	100–600	20	0	48	96	100	—	0.8	—	3.6	3
B. P.	100	60	*	*	*	*	—	2.0	3.4	—	2
J. B.	25	180	35	75	93	100	—	—	4.3	5.9	2
V. A.	25	180	93	—	—	—	2.4	—	—	—	1
V. S.	75	180	*	*	90	84	0.8	1.3	2.2	1.4	3
A. D.	150	10	49	100	—	—	—	—	—	—	1
G. R.	150	90	88	76	92	—	1.5	—	13.5	—	2

* Continued cortisone therapy interfered with eosinophil counts.

It is felt that this mode of administration is as yet an experimental one and should be used only when facilities for adequate laboratory evaluation of the changes produced are available.

As far as indications for intravenous ACTH therapy are concerned, they are apparent from the physiologic data discussed. In reactivating cortisone-inhibited adrenals, this mode of ACTH administration has proved most efficient (Table 6). The need for a particularly rapid and effective adrenal cortical stimulation, the saving in material, the occurrence of excessive intramuscular inactivation with certain types of ACTH preparations, and the desirability of applying an accurate dose of the hormone

represent the main advantages over the intramuscular route of ACTH administration.

DISCUSSION

An attempt has been made to study *quantitatively* the adrenal cortical response to ACTH in man, in order to establish its pharmacology. The intravenous route of administration insures the most direct contact of the hormone with the target gland and the use of a continuous infusion makes a constant, controlled stimulation possible. The choice of two indicators of adrenal activation was made on practical and theoretical grounds which have been discussed. *The fall in circulating eosinophils* served as a simple indicator of the rise in circulating 11,17-oxygenated adrenal steroids. *The rise in urinary 17-ketosteroids* has been assumed to represent an over-all measure of adrenal cortical response, at least in normal subjects. The increase in 17-ketosteroid excretion was found to be proportional to the logarithm of the dosage of ACTH employed, a finding in accordance with that of Luft, Sjogren and Li (30) using an ACTH polypeptide intramuscularly. Any state tending to change the transformation of compound F-like hormones into 17-ketosteroids would be expected to interfere with this relationship, as is the case for instance in the presence of liver disease (21). In spite of these well recognized limitations in relating changes in 17-ketosteroid excretion to differences in adrenal cortical activity, the relative ease of 17-ketosteroid determination made this the method of choice. However, with simpler procedures becoming available for the estimation of total 11,17-oxysteroids in urine (31, 32), this determination might prove to be a preferable indicator. Work along these lines is in progress.

Maximal adrenal cortical stimulation in the normal subject was obtained with the intravenous infusion of 20 I.U. of ACTH. This applies only to the standard eight-hour infusion, which was chosen on an empirical basis as the most effective and yet least inconvenient time period. Others have reported that in widespread debilitating diseases, such as pemphigus, up to 40 I.U. of intravenous ACTH may be required for control (22). A maximal rise in 17-ketosteroids in response to such a stimulus suggests that the steroid-discharging action of ACTH on the adrenal cortex is limited, unless one wants to assume the rather unlikely possibility that the metabolism of 17-ketosteroids is a limiting factor. It is of interest that the rectangular hyperbole which expresses the relationship of ACTH dose to the adrenal cortical response obtained, is identical with that obtained by Stadie when relating the effect of insulin concentration to the increased deposition of glycogen in the diabetic rat diaphragm in vitro (33). The shape of the curve was attributed by him to the limited number of absorption loci available to insulin, maximum action being obtained when all are used. Similarly one might conjecture that the degree of adrenal cortical activa-

tion produced by ACTH depends upon the surface area of the adrenal cortex available for ACTH action, maximum stimulation being obtained when all the available sites are being affected by ACTH.

The *time-response* relationship for ACTH suggests that only a fraction of the rapidly administered hormone is utilized for adrenal cortical activation. This phenomenon was first demonstrated by Sayers *et al.* (3) and subsequently by Greenspan, Li and Evans (34). A varying portion of the hormone is apparently inactivated and greater impurity of the material used results in more inactivation. Much appears to be held in the kidneys and destroyed there (35). Geschwind and Li have shown inactivation by liver, kidney, diaphragm and adrenal of both a large molecule and an active peptid fragment (9). ACTH of the type used in most of the present work appears to be inactivated by blood (36), in contrast to more highly purified preparations which are not so affected. This might explain the slight fall in eosinophils obtained with an impure preparation of ACTH, as opposed to a satisfactory fall in eosinophils following purer ACTH, when both were given in thirty seconds intravenously. By administering ACTH as a slow intravenous drip, fixation by the adrenal occurs continuously and before inactivation by other tissues has taken place to any significant extent. Hence the efficiency of the intravenous drip as opposed to massive intramuscular doses.

The eight-hour intravenous ACTH test is a more quantitative procedure than the intramuscular ACTH tests (37, 38), due to the lack of excessive local tissue inactivation which has been found to be a problem with certain types of ACTH. The single eight-hour intravenous test does not differentiate readily between primary adrenal insufficiency and that due to anterior pituitary ACTH failure. However, by running the test for three consecutive days a gradual increase in response will suggest a pituitary insufficiency, whereas failure will denote primary adrenal insufficiency. In instances of adrenal cortical involution, as shown in Figure 6, the much greater sensitivity of the eosinophils as opposed to the 17-ketosteroid excretion is clearly indicated. Whereas there is little danger of anaphylaxis in patients with normal adrenal cortical reserve, febrile reactions, chills and malaise may be occasionally encountered in those with a poor adrenal response. These manifestations are usually mild and it is anticipated that they will be less frequent with the increasing purity of available ACTH preparations. However, some untoward reactions have been reported (39).

Whereas a single infusion of ACTH will reveal the *immediate adrenal reserve*, composed presumably of pre-existing stored hormones and direct hormonal precursors, repeated daily infusions of ACTH will tend to reveal the *potential adrenal cortical reserve*, which is composed of the total synthetic capacity, as well as of the degree of functional and anatomic hyper-

trophy of which any given pair of adrenal cortices might be capable. Using 17-ketosteroids as an indicator, four distinct groups appeared among subjects given repeated daily infusions of ACTH. In one group a maximum was reached in two to three days. In a second group the rise in 17-ketosteroids occurred in two stages, the second rise being coincident with clinical improvement of widespread disease. This second rise might have been due to further improvement in adrenal cortical function or else to a change in the metabolism of 17-ketosteroids accompanying the control of the underlying disease. Decreased "utilization" of hormone at the periphery might be mentioned as a possibility. In a third group the excretion of 17-ketosteroids rose progressively throughout treatment and in the fourth group there was but a small rise of 17-ketosteroids in the absence of clinical signs of adrenal insufficiency. The final interpretation of these four groups must await similar studies in which the output of urinary corticoids, or better still the concentration of circulating 11,17-oxysteroids, is also determined. Only then will it be settled whether differences in adrenal response or changes in the metabolism of 17-ketosteroids are involved.

The occurrence of *resistance to ACTH* administered intramuscularly has been studied. The best explanation for the phenomenon observed would seem to be an increased fixation and destruction of ACTH at the site of injection. It is anticipated that increasing purity of the available ACTH will prevent the development of significant degrees of ACTH resistance (40).

The intravenous infusion of ACTH lends itself to *human assay*, using changes in 17-ketosteroid excretion as a measure of ACTH activity. The biometric limitations remain to be worked out in detail on a larger series.

As a form of therapy the intravenous administration of ACTH offers the advantage of economy and reproducibility with the disadvantage of many hours of immobilization. With increased purity, the majority of ACTH preparations now show practically identical potency by intravenous and intramuscular infusions.

SUMMARY AND CONCLUSIONS

1. The adrenal cortical response to the intravenous administration of ACTH has been determined quantitatively. Eosinopenia and the rise in urinary 17-ketosteroid excretion were used as indices of the degree of adrenocortical activation obtained.

2. For an eight-hour period of infusion the adrenocortical response obtained increased with the amount of ACTH used, up to a critical dosage over and above which no further response was elicited. In 8 subjects with normal adrenal function this critical dosage was 20 I.U. of ACTH, or less.

3. When a dose of 20 I.U. of ACTH was continuously administered for

periods varying from thirty seconds up to forty-eight hours, a linear increase in adrenal cortical stimulation occurred with increasing duration of administration. This suggests a rapid inactivation of excess ACTH, which appears to vary with the nature of the ACTH preparation used.

4. The use of an eight-hour infusion of 20 I.U. of ACTH intravenously as a test for the evaluation of the immediate adrenal cortical reserve has been illustrated.

5. Clinical patterns of response to a series of daily eight-hour infusions of 20 I.U. of ACTH intravenously have been presented as yielding some insight into the potential adrenal cortical reserve. The limitations in interpreting the results obtained have been stressed.

6. The marked metabolic effects of the prolonged, continuous intravenous infusion of ACTH have been presented.

7. Resistance to the action of intramuscularly administered ACTH following its previous use has been shown to occur with certain (somewhat impure) types of ACTH preparations. "Resistant" subjects were found to respond normally to the intravenous administration of the same material. Inactivation at the site of injection appears to be responsible for this type of "ACTH resistance."

8. The use of intravenous ACTH in the assay of the hormone in human subjects and in therapy has been documented by appropriate examples. It has been stressed that, using the intravenous infusion technique, microgram quantities of highly purified ACTH preparations may be tested for over-all metabolic activity in human subjects.

Acknowledgments

The authors are indebted to Dr. E. B. Astwood and Dr. J. B. Lesh for samples of purified corticotropin preparations and to Miss Nancy Grimes, Miss Claire Preston and Mrs. Lois Nellis for unfailing technical cooperation.

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STUDIES OF ELECTROLYTE METABOLISM IN 2 PATIENTS WITH PITUITARY INSUFFICIENCY*

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THE relative ease and accuracy of sodium and potassium determinations made possible by the introduction of the flame photometer a few years ago has led to intensified interest in the electrolyte metabolism in a variety of diseases. In this clinic, as elsewhere, investigators have been repeatedly confronted with the problem of edema formation in the presence of low serum electrolyte concentrations. This was first noted by us in 2 patients with Addison's disease. At the time of these observations, both patients were receiving added salt and desoxycorticosterone. It is well known that either of these agents in excess will produce edema in patients with this disease, but the simultaneous observation of low serum electrolyte levels seemed somewhat unusual. Furthermore, in spite of chloride levels as low as 80 milliequivalents per liter, neither patient showed signs of intravascular dehydration or circulatory inadequacy. Other clinics have reported low concentrations of serum electrolytes in certain cases of congestive heart failure (1, 2) and of cirrhosis of the liver (3, 4). The same phenomenon has been observed here and the administration of saline, even in hypertonic solution, has resulted primarily in the expansion of the extracellular fluid, without increasing the serum sodium concentration to normal levels.

Recently, in the course of treatment of a patient with hypopituitarism, edema formation became a therapeutic stumbling block while the serum concentrations of sodium and chloride were still far below normal. For this reason the patient was transferred to the metabolism ward, where electrolyte balance studies were carried out. The complicating factor of previous therapy caused some uncertainty in the interpretation of the results. Subsequently, a case of untreated "pituitary myxedema" was studied on the metabolism ward during a control period and while being treated with thyroid and later with testosterone propionate. The many hazards

Received for publication December 8, 1951.

* This investigation was supported in part by a research grant from the National Cancer Institute of the National Institutes of Health, Public Health Service; and institutional grants from the American Cancer Society and the Damon Runyon Memorial Fund for Cancer Research.

of interpretation of electrolyte balance data were all encountered, as well as the difficulties in serial biopsy determinations. The specific factors responsible for the persistence of low serum electrolyte concentrations in the presence of an abundance of total body sodium have not been entirely elucidated. Nevertheless, the analytic data are of considerable interest and perhaps may be helpful in suggesting future investigations in this field.

PATIENTS AND EXPERIMENTAL PROCEDURES

J.N. was a white male whose symptoms began in 1923 at the age of 25 with the gradual onset of cold intolerance and loss of body hair. By the age of 33 fatigue, pallor, dry skin and loss of libido were noted. He was first admitted to the Strong Memorial Hospital in 1933 (age 35) with findings which were thought to be typical of myxedema, including a basal metabolic rate of −35 per cent. He was given thyroid which he took for a period of approximately nine months. In February 1939 he was again seen in the clinic with full blown symptoms of hypothyroidism. Pulmonary infection was present at this time, as

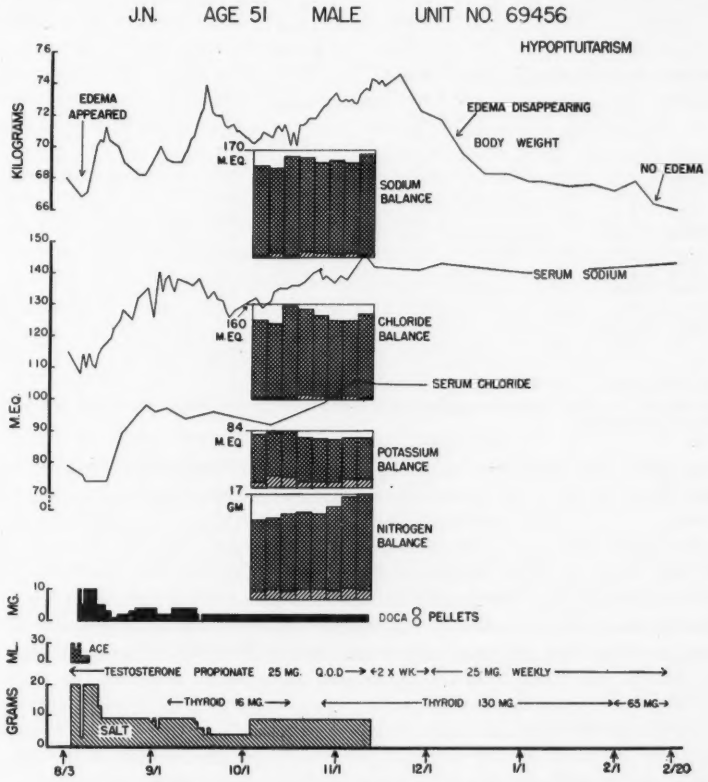

FIG. 1. Clinical course and balances (Patient J. N.).

well as evidence of electrolyte deficiency. The myxedema disappeared when thyroid was again given but an increased salt intake was necessary to correct the electrolyte disturbance. Six months later the results of a chloride excretion test (5) were within normal limits. It is of interest that adrenal insufficiency was manifest only in the presence of stress (pneumonia) at this stage. Although he discontinued all forms of therapy in 1942, he was not seen again in this clinic until August 1949 (age 51). His appearance at this admission resembled a state of hibernation. He was unresponsive, the skin was thin, dry and cold and there was no body hair. The blood pressure was 92/75; anemia was present; the BMR was −50 per cent; the serum sodium concentration was 116 mEq., chloride 80 mEq., carbon dioxide 28 mEq. per liter, and fasting blood sugar 61 mg. per cent. Stereoroentgenograms taken at this time, as well as in 1933 and 1939, showed the sella turcica to be normal. At first he was given 20 grams of salt daily; and after a few days on adrenal cortex extract he received desoxycorticosterone acetate in doses up to 10 mg. daily. Soon thereafter the latter was reduced to 2 to 4 mg. daily and the salt to 10 grams (Fig. 1). Testosterone propionate (25 mg.) was given every other day from the be-

TABLE 1. BALANCE DATA ON J.N.

Period, 5 days each	Initial weight, Kg.	Nitrogen			Potassium			Theoretical nitrogen balance from potassium, Gm. N per day	Sodium			Chloride		
		Urine, Gm. per day	Stool, Gm. per day	Balance, Gm. per day	Urine, mEq. per day	Stool, mEq. per day	Balance, mEq. per day		Urine, mEq. per day	Stool, mEq. per day	Balance, mEq. per day	Urine, mEq. per day	Stool, mEq. per day	Balance, mEq. per day
I	70.20	11.78	1.35	+4.25	68.7	11.6	+ 3.4	+1.2	141	1	+30	132	2.5	+26
II	70.71	12.06	1.57	+3.75	58.3	25.5	− .1	0	133	8	+30	128	2.6	+30
III	71.36	13.11	1.30	+2.97	63.6	23.4	− 3.3	−1.2	160	3	+ 7	157	2.0	+ 1
IV	71.36	13.12	1.53	+2.73	63.4	11.2	+ 9.1	+3.4	151	7	+12	146	6	+ 8
V	71.84	12.56	1.58	+3.24	59.7	11.7	+12.2	+4.5	144	6	+22	139	4	+17
VI	72.99	13.70	1.40	+2.28	61.4	9.7	+12.6	+4.7	148	4	18	131	2	+27
VII	72.98	15.51	1.76	+ .11	59.4	13.4	+10.9	+4.0	142	6	+22	129	4	+27
VIII	73.29 74.20	16.85	1.48	− .95	59.1	13.4	+11.2	+4.1	159	4	+ 7	144	2	+14

The daily intakes were: N 17.38 Gm.; K 84.7 mEq.; Na 170–172 mEq.; Cl 160 mEq.

Medications: DCA 2 mg. daily; testosterone propionate 25 mg. every other day; periods I and II, thyroid 16 mg. daily; periods VI, VII and VIII, thyroid 130 mg. daily.

ginning. Over a period of thirty days the serum sodium concentration rose to 140 mEq. per liter. Long before this level was reached, however, edema had developed. The extent of the edema varied with the amount of salt or desoxycorticosterone acetate administered. While the serum sodium level was approximately normal and he was receiving 10 grams of salt and 4 mg. of desoxycorticosterone acetate daily, thyroid was begun—at first 8, and later 16 mg. daily. The patient gained 6 kilograms in ten days and edema became a serious problem. Desoxycorticosterone acetate was decreased to 2 mg. daily and salt to 4 grams. As the edema regressed, the serum sodium level fell to 127 mEq. per liter.

The patient was transferred to the metabolism ward for study at this point. Since exacerbation of the edema coincided with the administration of thyroid, it was planned to determine the effects of administration and withdrawal of this substance, while other therapy was unchanged. The medications, which remained constant throughout the metabolic study, were a salt intake of 4 grams and 2 mg. of desoxycorticosterone acetate daily. Testosterone propionate, 25 mg., was continued every other day. For ten days

(Table 1 and Fig. 1, Periods I & II) a dose of 16 mg. of thyroid was given daily. It was then discontinued for fifteen days and following this, 130 mg. was administered daily for fifteen days (Periods VI–VIII). A constant diet of 2,405 calories was eaten. Analysis of this diet revealed 17.38 grams of nitrogen, 84 mEq. of potassium, 170 mEq. of sodium and 160 mEq. of chloride.

G.G. was a 65-year-old white male whose symptoms were of ten years' duration. Cold intolerance and moderate general apathy were first noticed. Two years later there was a gradual loss of body hair; he became impotent, and hot flashes were a prominent symptom. Four years before the present admission, signs and symptoms of increased intracranial pressure developed, and a chromophobe adenoma of the pituitary was removed. Following operation, the patient apparently led a life of invalidism with progressive weakness and lethargy. He was finally brought to the hospital in coma in October 1949. The patient had not been seen since the time of his operation and the only treatment given in the four-year interval was an occasional injection of testosterone propionate.

On admission his blood pressure was 150/100, he was unresponsive, the skin was fine and dry, and no body hair was present. There were scattered rhonchi throughout the chest. The left border of cardiac dullness was 12 cm. from the midsternal line and the heart sounds were distant. There was moderate edema of the extremities. From the extensive laboratory data, the observations on serum electrolytes proved to be of the most interest. Serum chloride concentration was 74 mEq., carbon dioxide, 25 mEq., sodium 106 mEq., and potassium 3.4 mEq. per liter. The blood sugar was normal. The patient was given rather large doses of salt (Fig. 2). The serum electrolyte concentrations rose, though not to normal levels. At the same time a weight gain of over 5 kilograms occurred. With reduction of the salt intake, the body weight dropped, as did the electrolyte levels.

It was felt at this time that it might be possible to carry out a balance regimen and the patient was transferred to the metabolism ward. In view of the edema, it was considered unwise to give desoxycorticosterone. Furthermore, we were interested in determining whether the electrolyte abnormality might be aggravated in this condition by treatment with thyroid, as has been suggested (6). After suitable control periods on salt alone, it was planned to give 130 mg. of thyroid daily. The diet was constant, containing 1,942 calories by calculation and 11.95 grams of nitrogen, 1.6 grams of phosphorus, 1.33 grams of calcium, 84 mEq. of potassium, 125 mEq. of sodium, and 135 mEq. of chloride, determined by repeated analyses. The sodium and chloride intake varied during the course of the study according to the amount of salt added to the basic diet.

METHODS

While being studied on the metabolism ward, both subjects consumed exactly the same food each day. The diets were weighed and cooked under the supervision of the dietitian and sample diets, duplicating in all respects the food served to the patients, were analyzed at least every ten days throughout the course of the experiments. Nitrogen, sodium, chloride, and potassium balances were carried out on both patients as follows: Urine was collected in 24-hour periods, analyzed daily, and the results checked on pooled aliquots, representing five days. Stools were pooled into corresponding 5-day periods, separation being accomplished with

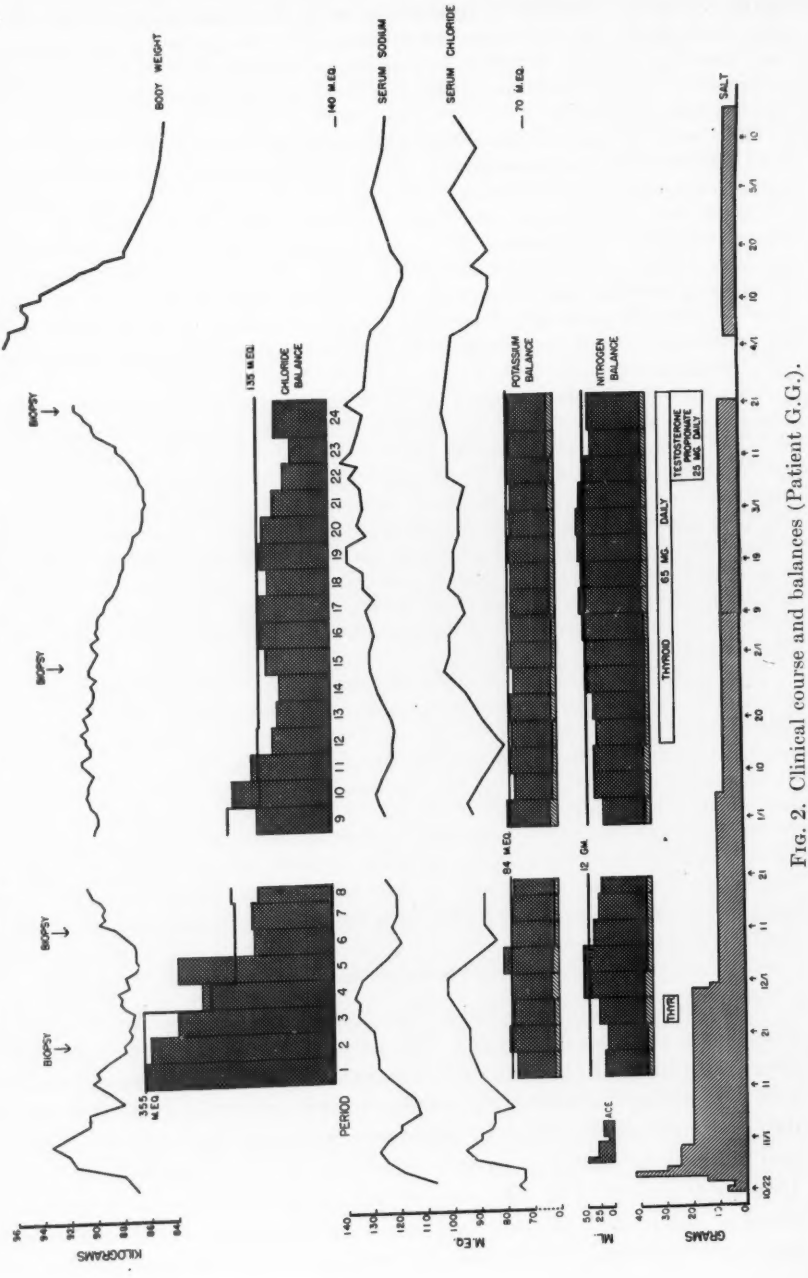

Fig. 2. Clinical course and balances (Patient G.G.).

carmine markers. On G.G., phosphorus balances were performed in the same manner, and the pooled specimens were used for the calcium balances. The analytic methods were the same as described previously (7). Three muscle biopsies were carried out on J.N. and four on G.G., the specimens being obtained under local anesthesia. The gastrocnemius and deltoid muscles were used. After wet and dry weights were obtained, fat was extracted with alcohol, ether and finally chloroform, in order to obtain the fat-free weight. Solvents were then evaporated and the residue ashed with the muscle, using nitric acid. Sodium, chloride and potassium determinations were carried out on the ash.

RESULTS

Clinical course

J.N. improved subjectively throughout the course of the 40-day experimental period. His myxedematous appearance vanished completely. He became alert and active. The only symptom which occurred during administration of 130 mg. of thyroid daily was soreness of the muscles for the first two days. Following the controlled study, the patient lost body weight slowly until all edema had disappeared. During this time the electrolyte concentrations remained normal (Fig. 1).

The clinical course of G.G. was somewhat more eventful (Fig. 2). Increasing cardiorespiratory difficulty reached alarming proportions during Period III and it became necessary to stop thyroid because of pulmonary congestion after five days of 130 mg. of the substance daily. The salt intake was reduced, but this proved ineffective in relieving the situation. He was therefore digitalized during Period V and this produced the desired diuresis. Digitalization was maintained throughout the rest of the experiment. Thirty-eight days were allowed to elapse before thyroid was again given (Period XII) in doses of 65 mg. daily. At this time moderate peripheral edema and pulmonary congestion were still present; nevertheless, the clinical response was favorable but slow. During the first sixteen days the weight did not decline, but the pulmonary congestion cleared gradually. The patient became more aware of his surroundings and complained only of an increase in the severity of hot flashes. The BMR which fluctuated from -25 to -30 per cent during control periods rose gradually to -7 per cent. After fifty days of thyroid, marked clinical improvement was observed. Testosterone propionate, 25 mg. daily intramuscularly, was then given as additional therapy (Periods XXII–XXIV). Pulmonary congestion and peripheral edema returned rapidly, necessitating the discontinuation of the metabolic study after fifteen days. Although no subsequent metabolic data were obtained, the patient was followed closely. After cessation of testosterone, he continued to collect edema fluid until he had

TABLE 2. BALANCE DATA ON G.G.

Period, 5 days each	Initial weight, Kg.	Nitrogen			Phosphorus			Calcium			Theoretical nitrogen from phosphorus and calcium, Gm. per day	Potassium			Theoretical nitrogen from potassium, Gm. per day	Sodium			Chloride		
		Urine, Gm. per day	Stool, Gm. per day	Balance, Gm. per day	Urine, Gm. per day	Stool, Gm. per day	Balance, Gm. per day	Urine, Gm. per day	Stool, Gm. per day	Balance, Gm. per day		Urine, mEq. per day	Stool, mEq. per day	Balance, mEq. per day		Urine, mEq. per day	Stool, mEq. per day	Balance, mEq. per day	Urine, mEq. per day	Stool, mEq. per day	Balance, mEq. per day
I	88.97	7.60	1.03	+3.32	.61	.51	+.48	.361	.767	+.14	+6.2	66.3	9.7	+8	+3	344	2	− 2	354	1	− 1
II	88.98	7.12	1.03	+3.80	.73	.51	+.36	.358	.767	+.20	+4.0	79.8	8.5	− 4.4	−1.6	342	1	+ 2	342	1	+ 12
III	87.32	8.24	1.58	+2.13	.93	.63	+.04	.311	.98	+.04	+0.3	70.4	9.9	+ 3.8	+1.4	286	4	+ 54	289	2	+ 63
IV	87.81	11.46	1.58	−1.00	.94	.63	+.03	.297	.98	+.05	+0.1	69.8	14.2	0	0	220	7	− 8	240	5	− 16
V	87.61	11.94	1.00	−0.99	1.24	.59	−.23	.341	.89	+.10	−4.0	87.1	12.8	−16	−5.9	307	1	−135	288	1	−106
VI	86.92	9.97	1.00	+ .95	1.17	.51	−.08	.426	.74	+.16	−2.3	76.5	7.8	0	0	112	.5	+ 60	145		+ 38
VII	88.38	8.81	1.00	+2.15	.98	.51	+.11	.466	.74	+.10	+0.9	70.6	7.8	+ 5.6	+2.1	132	1	+ 40	150	1	+ 32
VIII	89.45	7.70	1.00	+2.62	.86	.51	+.23	.466	.74	+.10	+0.9	64.7	7.8	+12	+4.3	119	1	+ 53	134	1	+ 53
	90.46																				
IX	89.87	7.07	1.28	+3.05	.89	.58	+.13	.456	1.00	−.13	+2.8	73.2	14	− 5	−1.9	127	2	+ 57	140	1	+ 54
X	90.09	8.66	1.21	+1.53	.83	.42	+.35	.354	.83	+.15	+4.2	61	10	+11	+4.1	181	3	− 61	183	2	− 50
XI	90.09	8.81	1.21	+1.58	1.16	.42	+.02	.383	.83	+.12	−0.5	72.6	7.6	+ 2	+ .7	141	1	− 15	150		− 13
XII	90.10	8.37	.77	+2.26	1.16	.36	+.08	.435	.80	+.09	+0.6	70.4	7.1	+ 4.5	+1.6	93	0	+ 34	108		+ 29
XIII	90.31	9.5	.77	+1.3	1.03	.36	+.21	.41	.80	+.12	+2.2	67.4	11.2	+ 3.4	+1.2	85		+ 40	101		+ 34
XIV	89.87	10.3	.77	+ .35	.96	.36	+.28	.34	.80	+.19	+2.9	61.2	11.2	+ 9.6	+3.5	79		+ 46	95		+ 40
XV	90.09	10.8	.77	− .19	.96	.36	+.28	.35	.80	+.18	+2.9	70	7.8	+ 4	+1.5	119		+ 6	121		+ 14
XVI	89.81	10.6	1.14	− .36	1.06	.40	+.14	.37	.91	+.05	+1.8	70	7.5	+ 6.2	+2.3	120	1.0	+ 4	129		+ 6
XVII	89.40	11.0	1.14	− .74	1.15	.40	+.05	.40	.91	+.02	+ .6	69	8.9	+ 5.6	+2.1	129	1.6	− 6	137		− 2
XVIII	88.17	10.7	1.14	− .48	1.07	.40	+.13	.38	.91	+.04	+1.6	69	9.2	+ 6	+2.2	109		+ 16	116		+ 19
XIX	87.43	11.56	1.00	−1.17	1.18	.42	−.02	.39	.93	+.01	−0.1	69.6	11.4	+ 3.4	+1.3	121		+ 4	129		+ 6
XX	86.67	11.77	1.00	−1.37	1.22	.43	−.05	.37	.93	+.03	−0.9	68	11.4	+ 4.6	+1.7	116	1.0	+ 8	126		+ 9
XXI	85.90	11.40	1.00	− .76	1.13	.42	+.04	.44	.93	−.04	+0.8	65	12.1	+ 7	+2.4	94		+ 31	106		+ 29
XXII	85.81	9.57	1.00	+ .43	1.05	.55	−.01	.24	1.08	+.01	− .1	74.4	10.5	− 1	− .4	61	2	+ 62	82		+ 53
XXIII	86.70	8.84	1.00	+1.56	.97	.55	+.08	.10	1.08	+.15	+ .1	65.4	13.1	+ 6	+2	65		+ 60	73		+ 66
XXIV	88.51	9.22	1.00	+1.18	.93	.55	+.12	.06	1.08	+.19	+ .4	67	12	+ 5	+1.9	79	3	+ 45	99		+ 36
	90.88																				

Period III, thyroid 130 mg. daily; period IV, salt intake reduced; period V, digitalization; period X, further reduction of salt; period XII, thyroid 65 mg. daily begun; periods XXII–XXIV, testosterone propionate 25 mg. daily.

For intake: see text; or add urine, stool and balance.

gained 10 kilograms of body weight. Diuresis seemed indicated and was produced with Mercuhydrin. The serum electrolyte concentrations fell as the diuresis occurred (Fig. 2 after Period XXIV).

The patient was seen at weekly intervals during the next eight months. He was maintained on a low salt diet (3 to 4 grams daily), digitalis, thyroid (65 mg. daily) and 25 mg. of testosterone propionate weekly. He was ambulatory and complained only of pains in his legs with exercise. Serum electrolyte levels were consistently low—sodium 116 to 122 and chloride, 84 to 94 mEq. per liter. One attempt to allow a more liberal salt intake promptly resulted in a weight increase of 3 kilograms.

In January 1951 he was readmitted for a trial of adrenal cortical extract. During the preliminary control period he suddenly expired of a coronary occlusion. Aside from the myocardial infarction, the important autopsy findings concerned the endocrine system. There was replacement of almost the entire pituitary by fibrous tissue. A very few cells of probable posterior pituitary tissue were seen. The adrenals were small, measuring $3 \times 0.5 \times 0.5$ cm. The cortical zone was thin and the fasciculata shrunken. All tissues showed eosinophilic infiltration.

Nitrogen balance

Both patients were in positive nitrogen balance during the uncomplicated control periods (Table 1, Fig. 1; Table 2, Fig. 2) and were storing between two and three grams a day. Mobilization of salt by reduction of the salt intake and by digitalization produced a temporary negative balance in G.G. (Periods IV and V). The time relationship seems to indicate that the nitrogen loss was associated with the salt loss (8), rather than with the increased work of dyspnea. The latter was worse during Period III, while nitrogen loss occurred during Periods IV and V when the pulmonary congestion had improved.

When 130 mg. of thyroid was given daily to J.N., the urinary nitrogen increased rapidly, nitrogen equilibrium was reached in ten days and a negative nitrogen balance became established at the end of fifteen days (Table 1, Fig. 1, Periods VI–VIII). When the smaller dose (65 mg.) of thyroid was given to G.G., beginning with Period XII, the urinary nitrogen rose more slowly and equilibrium was reached only after twenty days. Even after fifty days the negative balance was not pronounced, being in the neighborhood of 1 gram daily. The net loss of nitrogen over the fifty days was 4 grams.

Fat balance

The caloric balances of the 2 patients were calculated according to the method of Newburgh (9). It was realized that certain discrepancies might

be encountered since it is known that the insensible water loss may be slightly low in comparison to heat production in myxedema and high in congestive heart failure. The figures obtained are considered to be more significant for comparative than for absolute values. In J.N. (Table 3)

TABLE 3. FLUID AND ELECTROLYTE EXCHANGE OF J.N.

Period	Weight change, Gm.	Dry protein, Gm.	Fat, Gm.	Extracellular fluid, Gm.	Intracellular sodium, mEq.	Remarks
I	+ 510	+133	+463	+1070	+35	Thyroid 16 mg.
II	+ 650	+118	+506	+680	−63	Thyroid 16 mg.
III	0	+92	+373	−130	+12	No thyroid
IV	+ 480	+95	+278	−900	+97	No thyroid
V	+1150	+101	+485	+650	+23	No thyroid
VI	− 10	+71	+271	+830	−50	Thyroid 130 mg.
VII	+ 310	+3	+179	0	+11	Thyroid 130 mg.
VIII	+ 910	−30	+410	+520	−45	Thyroid 130 mg.

Note: the extracellular fluid at the beginning was assumed to be 15.8 liters, or 22.5 per cent of the body weight.

Periods: five days each.

during the first five periods (16 mg. of thyroid for ten days followed by no thyroid for fifteen days) there was a positive caloric balance with a fat storage of about 80 grams a day, a reasonable figure considering the intake (2,405 calories) and activity of the patient. When 130 mg. of thyroid was given, the rate of fat storage decreased rapidly, reaching a low of 30 grams daily ten days after thyroid was begun. The calculated increase in fat storage during Period VIII is unexplained. The muscle biopsy specimens contained 1.9 and 4.3 per cent fat.

The other patient, G.G., on an intake of about 1,900 calories, was in negative caloric balance throughout the experimental period. During the control periods (I–II, IX–XII) this amounted to a fat loss of about 40 grams daily (Table 4). During the fifty days of thyroid administration, the fat loss gradually but steadily increased, reaching 100 grams a day after five periods of five days each and 168 grams a day at the end of ten periods, of fifty days. The analytic values of fat in the biopsy specimens are quite likely more than coincidental. The first two specimens, both in control periods, contained 8.5 per cent and 7.8 per cent fat; the third, sixteen days after thyroid was started, 5.9 per cent; and the fourth, at the termination of the experiment, 2.8 per cent.

TABLE 4. FLUID AND ELECTROLYTE EXCHANGE OF G.G.

Period	Weight change, Gm.	Dry protein, Gm.	Fat, Gm.	Extracellular fluid, Gm.	Intracellular sodium, mEq.	Remarks
I	−1290	+103	− 249	−1234	+ 14	
II	−2020	+118	− 471	+ 113	− 72	
III	+ 850	+ 66	− 223	+ 600	− 26	Thyroid 130 mg./day
IV	− 200	− 31	−1049	− 328	+ 52	Salt reduction
V	− 680	− 31	+ 67	−2500	−127	Digitalis begun
VI	+1455	+ 29	− 134	+ 600	+153	
VII	+1070	+ 67	− 209	+1150	+ 7	
VIII	+1010	+ 49	− 76	+1250	− 24	
IX	+ 480	+ 59	− 222	+1780	− 20	
X	0	+ 48	− 91	−1130	−148	
XI	+ 10	+ 49	− 316	+ 200	− 65	
XII	+ 210	+ 71	− 208	− 700	+206	Thyroid 65 mg./day begun
XIII	− 440	+ 42	− 256	− 250	+ 62	
XIV	+ 220	+ 11	− 285	− 150	+126	
XV	− 280	− 6	− 333	+ 130	− 49	
XVI	− 480	0	− 314	− 180	−156	
XVII	−1230	− 7	− 500	− 450	− 63	
XVIII	− 740	− 15	− 614	+ 850	− 94	
XIX	− 760	− 37	− 676	+ 250	− 41	
XX	− 770	− 43	− 795	− 50	− 31	
XXI (4 days)	− 90	− 19	− 669	+1100	− 78	
XXII	+ 890	+ 13	− 682	+ 500	+ 8	Testosterone prop. 25 mg./day
XXIII	+1810	+ 49	− 985	+2400	+ 67	Testosterone prop. 25 mg./day
XXIV	+2370	+ 50	− 800	+1500	−130	Testosterone prop. 25 mg./day

Note: the extracellular fluid at the beginning was assumed to be 22 liters, or 25 per cent of the body weight.

Calcium and phosphorus balances

These balances were carried out only on the second of the 2 patients (Table 2). By themselves, the figures point to only one abnormality, that of a high urinary calcium excretion, 300 to 350 mg. a day on an intake of 1.33 grams. Thyroid increased this excretion slightly to a peak of 400 mg. Testosterone propionate, of course, reduced it to a low of 50 mg. In spite of the high urinary excretion the balance was at all times positive.

Of the greatest interest in these balances is the theoretical nitrogen derived from the phosphorus and calcium balances (10). During the fifty-five days of control the actual nitrogen balance was positive by 94 grams; the theoretical nitrogen was positive by only 59 grams. The situation was reversed when thyroid was given, the patient having a net loss of nitrogen during these fifty days of 5 grams, while the theoretical nitrogen from phosphorus was still strongly positive, *i.e.*, +60 grams. This discrepancy of the nitrogen-to-phosphorus ratios suggests the storage of nitrogen without the usual quota of phosphorus during control periods. It also suggests that subsequently, under the influence of thyroid therapy, there was loss of nitrogen from phosphorus-poor tissues. The other possibility for the last discrepancy was glycogen formation. This seems quantitatively unlikely because of the magnitude of the phosphorus retention (127 milliosmols).

Sodium and chloride balances

A skin loss of 4 mEq. of sodium and chloride daily was estimated for J. N. from data during control periods (11). When this was assumed to be constant, the sodium chloride balances of J.N. were found to be positive throughout the experiment, with the exception of the first two periods after thyroid was withdrawn (Periods III & IV, Table 1, Fig. 1).[1] Part of the retention was accounted for by a steady rise in the concentration of extracellular sodium and chloride (4.8 and 7 mEq. per liter, respectively); the remainder was considered to be associated with a moderate increase in extracellular space. Variations in the volume of the latter were calculated from the chloride balances, after making the proper corrections for changes in the chloride level (12). The changes which were estimated to have taken place in the sizes of the various body compartments during each period are listed in Table 3 and the cumulative effects are represented graphically (Fig. 3). It was thought that the effects of desoxycorticosterone acetate and testosterone propionate were responsible for both the steady increase in volume of extracellular fluid and rise in concentration of electrolytes therein. Any effect of thyroid on electrolyte metabolism was evidently overshadowed by that of the other hormones. Calculated changes of intracellular sodium were so small that they probably fall within the limits of error.

The sodium and chloride balances of G.G. (Table 2) are worthy of further comment. The skin loss was estimated at 10 mEq. daily and considered constant. As in J.N., after proper allowances had been made for changing serum levels, the chloride balances were considered to reflect changes in the extracellular space and these are recorded in Table 4. At

[1] In Tables 1 and 2 and Figures 1 and 2, no corrections for skin losses were made.

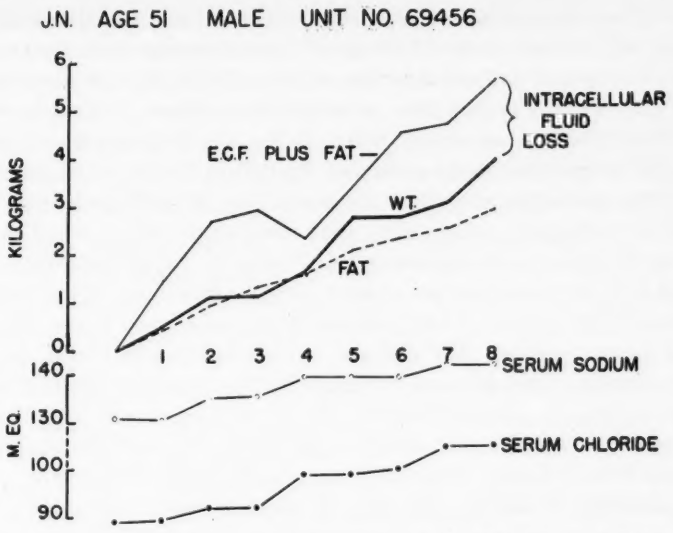

FIG. 3. Cumulative changes in body compartments in J.N. during balance studies.

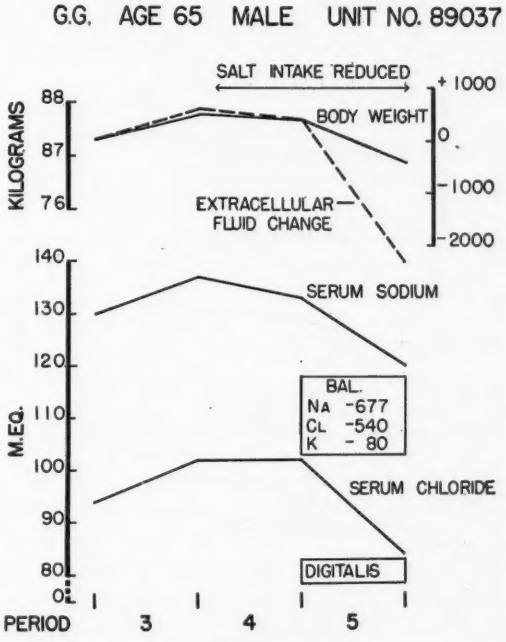

FIG. 4. Effect of diuresis after digitalization on concentration of serum electrolytes and on body fluids in Patient G.G.

the end of ten control days, the patient was essentially in salt equilibrium. In Period III, when a dose of 130 mg. of thyroid was given daily there was a large retention of sodium chloride, accounted for in large measure by a sudden rise in the concentration of serum electrolytes. A small increase of extracellular fluid also occurred (600 cc.) (Fig. 4). Evidence developed that the cardiac reserve had been exceeded. In Period IV the administration of thyroid was discontinued and on the second day the salt intake was halved because of pulmonary congestion. This resulted in only a small salt loss. When digitalization was carried out in Period V, about 500 mEq. of chloride and even more sodium were lost during the five days. The result was a decrease of over 2 liters of extracellular fluid and a drop in the concentration of serum sodium and chloride of about 12 mEq. each per liter. Six periods were then allowed to elapse for the action of thyroid to wear off and to obtain reasonable control data. It was necessary to decrease the salt intake still more in order to accomplish this. Thyroid was then started again at a dose of 65 mg. a day, beginning with Period XII (Fig. 5). Significant quantities of sodium and chloride were retained for the next fifteen

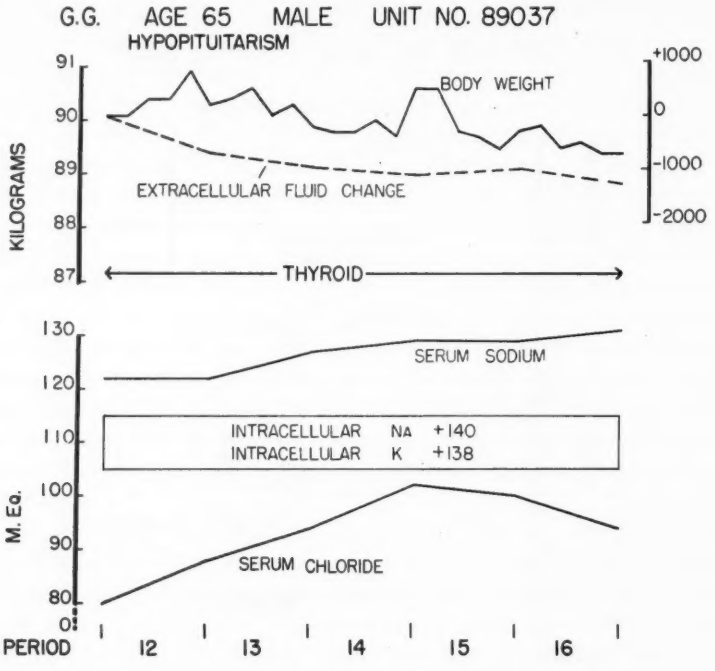

FIG. 5. Serum electrolyte and body fluid changes after thyroid administration (Patient G.G.).

days (450 and 365 mEq.). This resulted in a dramatic rise of the extracellular electrolyte concentration; the serum chloride level increased 20 mEq. and sodium 10 mEq. At the same time about a liter of extracellular fluid was lost. During the next thirty-five days, the salt balance was slightly negative for the greater part of the time; the serum electrolyte concentrations remained stationary, though the sodium level was still below normal (about 135 mEq.). Finally, in the last three periods, testosterone propionate

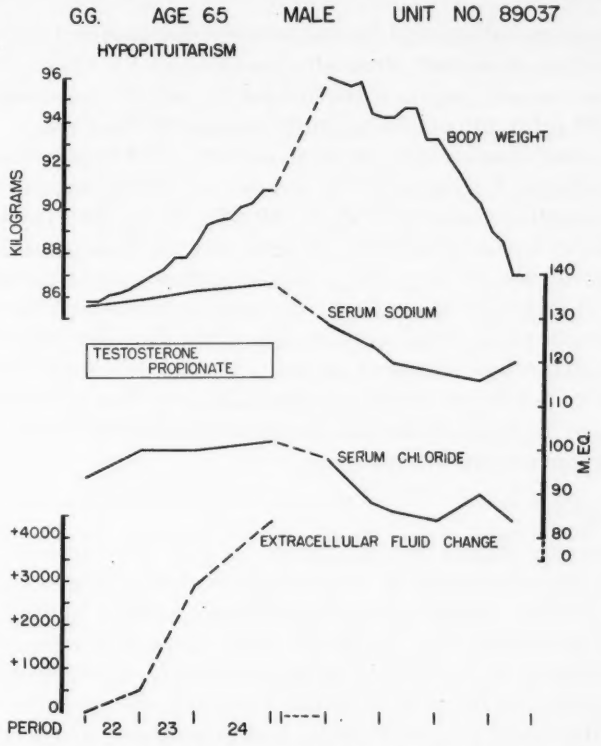

FIG. 6. Serum electrolyte, extracellular fluid and weight changes in G.G. after administration of testosterone propionate.

was given, associated with a massive retention of extracellular fluid (Fig. 6). As stated before, this caused the cessation of the balance studies at the time.

The calculated changes of intracellular sodium were of sufficient magnitude at four times during the experiment to be of significance (Table 4). During the diuresis produced by digitalization (Period V) and by reduction of the salt intake (Period X), sodium appears to have been lost from the cells. During the first fifteen days of thyroid administration (Periods

XII–XIV), it was calculated that there was a gain of sodium in the cells; but this and more was lost in the subsequent forty-five days. It is interesting to note that during the periods of sharp fall in extracellular electrolyte concentration, sodium was probably withdrawn from the cells; and in contrast, when an extracellular rise in concentration occurred, as initially with thyroid, the intracellular concentration of sodium must have risen.

Potassium balance

The potassium balance in J.N. was not remarkable except in one respect. During the time of thyroid administration (Periods VI–VIII), as the nitrogen balance became negative the potassium balance remained strongly positive, 175 mEq. of potassium being retained at this time.

In G.G. three observations are worth noting: 1) the large losses of sodium chloride in Period V (digitalization and salt reduction) were accompanied by a large negative potassium balance, 80 mEq. being lost (Table 2) during the five days; 2) the theoretical nitrogen derived from potassium during the first eleven periods was +21 grams in contrast to the actual nitrogen balance of +94 grams; 3) as in J.N., thyroid administration was associated with a strongly positive balance throughout the fifty-day period while the nitrogen balance was becoming negative (+93 grams for the theoretical, as opposed to −4 for the actual nitrogen balance). This has been observed previously by Byrom (13) and was interpreted as evidence that extracellular protein was present in myxedema.

Muscle biopsies

Three muscle biopsies were performed on J.N. and four on G.G., in the hope that the concentrations of electrolytes therein might be correlated with the observed balances. This hope was not entirely fulfilled, primarily because of the uncertainty about the exact amount of extracellular fluid in each specimen. It is usually thought that there is very little or no chloride in muscle cells, and the amount of this halogen in the specimen divided by the concentration in the extracellular fluid is assumed to be a fair estimate of the extracellular volume. The hazards of this assumption in small biopsy specimens such as these (between 1 and 2 grams) become obvious as one examines the analytic data. It is possible that even with careful technique in handling the specimens and removal of excess blood, the greatest source of error is the chloride from the red blood cells which remain in the specimen.

The first muscle biopsy specimen was removed from J.N. at the beginning of the second period, the next was taken thirty days later and a third was taken twenty-one months after completion of the metabolic study. He was then in good clinical condition, had normal levels of serum electro-

lytes and was free of edema. In G.G., biopsy specimens were taken on the last day of the second control period; twenty-one days later, during Period VII; sixteen days after thyroid was begun (Period XV); and again at the end of the experiment (Fig. 2). The analytic results and various calculations therefrom are presented in Table 5.

TABLE 5. MUSCLE BIOPSIES

	Per Kg. fat-free tissue								Per 100 Gm. fat-free solids	Per L. intracellular water		
	Fat, %	Water, Gm.	Solids, Gm.	Chloride, mEq.	Sodium, mEq.	Potassium, mEq.	Extracellular water, ml.	Intracellular water, ml.	Potassium, mEq.	Sodium, mEq.	Potassium, mEq.	Potassium and sodium, mEq.
J. N. 1	1.9	788	212	17	42	85	167	621	41	32	131	162
2	4.3	800	200	34	51	80	292	508	40	18	155	173
3	3.6	783	217	32	37	86	280	503	40	0	170	170
G. G. 1	8.5	792	208	33	48	80	310	482	38	15	165	180
2	7.8	807	193	18	42	74	187	620	38	36	120	156
3	5.9	791	207	24	43	75	207	582	36	27	129	156
4	2	767	233	30	50	76	260	507	33	27	150	177
Normal (4) Average		791		33	36	89	292	499	43	0	173	
Range						82–93			41–45		170–176	

The sodium determinations on the 6 specimens taken during the metabolic study (*i.e.*, all except specimen #3 on J.N.) ranged from 42 to 51 mEq. per kilogram of fat-free muscle, in contrast to 36 mEq. obtained on normal biopsy material. When the amounts of extracellular fluid listed in the table were assumed to be present, it was found that the concentration of sodium in the cell fluid was from 15 to 36 mEq. per liter. It should be remembered that the errors involved in determining extracellular fluid, previously discussed, are such that these figures for intracellular concentration of sodium, as well as the estimated abnormalities of potassium concentration, are probably conservative.

In the same 6 specimens the potassium per kilogram of fat-free muscle tended to be low, with the exception of the first on J.N. When calculated with reference to fat-free solids, only the specimens from G.G. can be considered definitely abnormal. The calculated potassium concentration per liter of intracellular fluid was lower than our values obtained on 4 normal control subjects in all specimens, with the exception of the first on G.G. and the third on J.N. (the latter taken when the patient was clinically well). It is suspected that the normal value for the first specimen on G.G. may be in error, since calculated values were in disagreement with clinical

condition, body weight and balances. It should be noted that our control value for potassium concentration is higher than that found elsewhere (14), a discrepancy we cannot explain. All that can be said is that the specimens taken from these patients were prepared and analyzed in exactly the same manner as the control specimens.

In J.N., fairly good qualitative agreement was found between the intracellular potassium concentration in the biopsy specimens, the balances, and his clinical condition. It will be noted in Table 5 that during the metabolic experiment the intracellular fluid was thought to have decreased in content of sodium, while there was increase in content of potassium. At the same time the patient was in positive caloric, nitrogen and potassium balance. Soon after the balance study he lost edema spontaneously and the third biospy specimen, taken twenty-one months later, was essentially the same as the normal control biopsy specimens.

DISCUSSION

Low serum electrolyte concentrations with contraction of the plasma volume, depression of glomerular filtration, and circulatory collapse are commonly seen in salt deficiency. Recently there have been reports of low serum electrolyte concentrations in the absence of obvious intravascular dehydration, accompanying a rather wide variety of chronic diseases. This phenomenon has been observed in far advanced tuberculosis (15), in tuberculous meningitis (16), in hepatic cirrhosis (3, 4) and in cardiac decompensation (1, 2). Only when the underlying disease process itself has been controlled, has the serum electrolyte concentration been restored to normal levels.

In the course of the study of our patients, numerous complications were encountered. The exact sequence of events was not always evident and the interpretation of the etiologic factors involved may allow considerable controversy. Certain characteristics of the electrolyte aberrations were, nevertheless, striking and definite.

1) Both patients showed low serum electrolyte concentrations over long periods of time, even while there was no evidence of a deficit of total body sodium and chloride.

2) Excessive retention of extracellular fluid, resulting in edema, readily occurred in the presence of persistently low concentrations of serum sodium and chloride. When the retention of sodium and chloride was produced by a high salt intake or the administration of desoxycorticosterone acetate or testosterone propionate, the levels of sodium and chloride in the plasma were increased but the extracellular fluid underwent still further expansion.

3) Evidence of excessive quantities of sodium and deficient amounts of potassium within the cells was obtained by both metabolic balance data and muscle biopsy.

4) In neither patient was the increased nitrogen excretion observed during therapy with thyroid substance accompanied by the loss of potassium and phosphorus in the quantities usually associated with the destruction of protoplasm. This observation has usually been interpreted as evidence that in myxedema an extracellular mucoprotein is present, which is liberated under the influence of thyroid substance (17). The thyroid hormone may also serve as a stimulus for glycogen formation with additional potassium retention.

The low concentrations of electrolytes in the plasma of these patients must mean that the osmotic pressure within the cells, as well as that of the extracellular fluid, was less than normal. It is generally accepted that changes in osmotic pressure will cause alterations in the volumes of the three fluid compartments of the body. In a state of equilibrium the osmotic pressures of the intracellular and interstitial fluids must be equal. That of the circulatory fluid is higher by an amount approximately the same as the hydrostatic pressure, the greater osmotic pressure being due to the presence of the plasma proteins. Changes of these forces within the vascular system and in the resistance furnished by the tissues are responsible for the movements between the vascular bed and interstitial spaces. The variables responsible for the shift of water between the extracellular compartment and the interior of the cell are less well understood. It is usually thought that the osmotic pressure within the cells is adjusted to that of the interstitial fluid by shifts of water. This has been accomplished by changing the osmotic pressure of the extracellular fluid artificially (18). Such a shift must also take place in certain clinical situations in which there has been loss of electrolytes through the gastro-intestinal or genito-urinary tracts. In many conditions, however, one wonders whether the metabolic status of the cells may not be largely responsible for the osmotic pressure therein, and that the extracellular fluid must be adjusted accordingly.

Since these patients had obvious multiple endocrine deficiencies, the role of the endocrines in electrolyte and water metabolism deserves detailed consideration, especially with respect to the possibility that a primary reduction of the tonicity of the extracellular fluid may result in reduction of the osmotic pressure within the cell. In both our patients disturbances due to adrenal insufficiency were to be expected. Two well known phenomena have been described in the absence of these glands. There is inability to conserve salt, which is thought to be due to failure of the renal tubules to reabsorb sodium. This is the basis for the test devised by Cutler, Power and Wilder (5). Replacement can be accomplished with the administration of salt-retaining hormones or large doses of salt, and the concentration of serum sodium will usually reach normal levels before the extracellular fluid is abnormally expanded. Failure to excrete a water load in normal

fashion is the other abnormality which has been studied extensively (19) and is the basis for the first part of the Robinson, Power and Kepler test (20). This failure may be secondary to the antagonistic relationship which exists between the antidiuretic hormone of the posterior pituitary (ADH) and the adrenal-anterior pituitary axis. Nagareda and Gaunt (21) have expressed the opinion that the integration of these hormones may be achieved in hydrated states by a converse response of the adrenals and the posterior pituitary to changes in the osmotic pressure of the plasma. With deficiency of the adrenals, then, one might expect unrestrained activity of ADH and perhaps excessive retention of water. Recently Lloyd, Harris and Lobotsky (22) have reported that Pitressin caused increased excretion of sodium and retention of water in some patients with liver disease. The resultant rearrangement of the extracellular fluid pattern in such patients would eventually cause intracellular electrolyte changes. One would expect that such dilution might reduce all intracellular components proportionately. This may have been the case in J.N., but in G.G. the potassium was reduced more than the solids (Table 5). It is difficult to understand why the ingestion of adequate quantities of potassium and sodium should not correct the osmotic defect, unless some cellular injury was present. Furthermore, perpetuation of the osmotic defect in this manner would probably require a revision of the hypothesis, which is currently accepted, that ADH production is increased by increasing the osmotic pressure of the extracellular fluid. Leaf and Mamby (23) have reported the presence of an antidiuretic hormone in the blood when solute concentrations therein were low. Such an agent could explain the inability of our patients to excrete their excessive amounts of extracellular fluid. The process which initiated the low solute concentration, however, would still require explanation. If it is the ADH of the posterior pituitary, then we must postulate from our data that ADH disturbs the composition of all body cells or the permeability of their membranes and that renal aberrations are some of the manifestations thereof. We should point out in this connection that the autopsy of G.G. revealed probable total destruction of the posterior pituitary, although it must be admitted that the supraoptico-hypophyseal system above the stalk may have been intact and capable of secreting ADH (24).

Ample evidence of other hormonal and metabolic derangement was present in both patients. Deficiencies of thyroid and androgenic activity were obvious. That myxedema alone may cause aberrations of the serum electrolyte pattern as well as of water exchange is suggested by the work of Statland (25) and Knowlton (26). Not only have low serum electrolyte concentrations been seen occasionally in myxedema, but failure to excrete an excessive load of electrolytes and water has also been observed. It has

been suggested that these findings are the result of myxedematous infiltrations in the adrenals (25), and their disappearance with the administration of thyroid substance has been reported. That the defect in electrolyte metabolism was improved by thyroid substance in these patients is probable. The failure of G.G. to maintain a higher serum osmotic pressure while receiving a hormone causing electrolyte retention (testosterone propionate) in addition to thyroid substance, suggests that the fundamental abnormality responsible for the low osmotic pressure still existed.

The discrepancy between the nitrogen balance and the phosphorus and potassium balances during thyroid therapy may have been related to the release of an extracellular mucoprotein but it could also be explained by the presence of protein within the cells which was not associated with the amounts of phosphorus and potassium found in normal protoplasm. These findings, then, might represent additional evidence for a primary intracellular metabolic change. Such a change could result in a low intracellular osmotic pressure which would in turn be reflected in the extracellular fluid.

If, as has been suggested by Brock (27), the base equivalence of proteins may vary, then the amount of protein within the cell may be normal or high and the electrolyte concentration and osmotic pressure low. This would result in a reduction in the electrolyte concentration outside the cells and perhaps a tendency to form edema with any effort to raise the extracellular concentration of solutes. For example, we may assume that G.G. (whose normal weight was 75 Kg.) had 15 liters of extracellular fluid and 37.5 liters of intracellular fluid with a normal osmotic concentration of 310 milliosmols per liter, before the onset of his illness. He then had: 4,650 (310×15) milliosmols in his extracellular fluid and 11,625 (310×37.5) milliosmols in his intracellular fluid.

If it is assumed that because of his illness the base-binding power of the proteins associated with 1 liter of intracellular water decreased by approximately 14 mEq., he lost 525 mEq. of potassium from his cells, leaving $11,625-525=11,100$ milliosmols. The subsequent decrease of osmotic pressure outside the cells, essential for the establishment of equilibrium among the compartments of the body water, could have been accomplished in several ways, namely: excretion of excess electrolyte from intracellular and extracellular sources, retention of ingested fluid, or redistribution of water present in the body. If the liberated potassium and the proportional amount of extracellular electrolyte were excreted, the body compartments might maintain their volumes with decreased osmotic pressure. The retention of ingested water in amounts necessary to produce the reduction in extracellular osmotic pressure would obviously cause expansion of the extracellular fluid. If the potassium released from the cells were excreted

but the excess extracellular sodium were retained, the extracellular fluid would have increased to 15.5 liters ($4{,}650/15+x=11{,}100/37.5-x$). Although the latter two situations would explain some increase in extracellular fluid, the exaggerated retention of sodium in these patients when efforts were made to increase the concentration of sodium in the extracellular fluid, must mean that a renal factor was present. The increased renal reabsorption might result from a decrease in serum concentration alone. The biopsies as well as the results of the metabolic balance studies showed furthermore, that with this expansion of extracellular fluid, sodium had been forced to enter the cells. It is important to remember that during this time both patients were ingesting amounts of potassium (85 mEq. daily) which are usually adequate to permit retention within the body.

It is suggested that a primary intracellular metabolic defect was present in both patients and that this was responsible for the reduced osmotic pressure. Gradual restitution of both the intracellular and extracellular phases occurred as the disease process was controlled in J.N. This response was probably associated with the positive caloric, nitrogen and potassium balances. The latter two may have been potentiated by the androgen therapy. The failure of G.G. to attain normal electrolyte concentrations during the period of observation may have been due to inadequate therapy or less than optimal caloric intake during therapy.

SUMMARY

Balance studies for 40 and 120 days respectively were carried out on 2 patients with pituitary insufficiency. Both patients had low serum electrolyte concentrations over long periods of time, even while there was no evidence of deficit of total body sodium and chloride. Excessive retention of extracellular fluid, resulting in edema, readily occurred in the presence of persistently low concentrations of serum sodium and chloride. There was evidence, by muscle biopsy, of excessive quantities of sodium and a deficiency of potassium within the cells. It was felt that the most likely explanation lay in a defect of intracellular metabolism, which was responsible for a lowered osmotic pressure within the cell; and that the reduction in extracellular electrolyte concentration was secondary to this defect.

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NEUROGENIC HYPERNATREMIA AND HYPERCHLOREMIA

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IT IS the purpose of this report to bring attention to the not infrequent occurrence of unusual electrolyte disturbances associated with disease of the central nervous system. The electrolyte abnormalities with which this paper is particularly concerned are the development of marked hypernatremia, hyperchloremia, and hypochloruria during the course of a disease involving primarily the brain. The unusual electrolyte disturbances in these cases can not be satisfactorily accounted for on the basis of our present knowledge of the control of electrolyte metabolism. Accordingly, it is considered worthwhile at this time to review those cases which have been reported to date and to report briefly 4 additional cases.

REVIEW OF LITERATURE

The first report dealing specifically with hypernatremia and hyperchloremia associated with cerebral lesions, in the absence of renal disease, was that of Allott in 1939 (1). This author presented 5 cases in which such electrolyte abnormalities were noted during the course of fatal disease of the brain. Allott's cases demonstrated hypernatremia, hyperchloremia and azotemia associated with a markedly decreased urinary excretion of sodium and chloride. However, there was an elevated urinary excretion of potassium, phosphate and sulphate ions. The pathologic processes of the cerebrum in 3 of these cases were ruptured aneurysm of the anterior cerebral artery, recent cerebral infarct, and tumor of the choroid plexus, respectively. Sweet and associates (2) reported a case of hypernatremia, hyperchloremia, hyperglycemia, hypokaliemia and gastro-intestinal hemorrhages following a prefrontal lobotomy in a 47-year-old woman who died on the thirty-second day after operation. This case was carefully studied and excellently reported. These authors also mentioned 3 additional cases of bizarre electrolyte abnormalities associated with neurologic disease. Biemond (3) has described a syndrome of intermittent oliguria with edema of the face secondary to a hypothalamic lesion in a 32-year-old woman.

Received for publication December 27, 1951.

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This author pointed out that the only electrolyte abnormality was elevation of the level of serum sodium to 155 mEq./liter.

Leutscher and Blackman (4) have reported hypernatremia and hyperchloremia associated with cerebral lesions and renal degeneration in 5 cases of sulfonamide intoxication. Each of these patients had impairment of consciousness, mental abnormalities, or focal neurologic signs. In view of the advanced renal lesions, the relation of these unusual electrolyte disturbances to the cerebral lesions was not as striking as in the previously cited cases. Although worthy of note and germane to the present report the cases of Leutscher and Blackman are not included in Table 1 because of the associated renal lesions. However, Sweet *et al.* have already postulated that the electrolyte abnormalities in these cases may have been secondary to the cerebral rather than the renal lesions.

In collaboration with MacCarty, one of us (I.S.C.) has previously reported 3 cases of hypernatremia, hyperchloremia and hypochloruria as-

TABLE 1. TABULATION OF 18 REPORTED CASES OF HYPERNATREMIA AND/OR HYPERCHLOREMIA ASSOCIATED WITH CEREBRAL LESIONS INCLUDING CASES IN THIS REPORT

Author	Case number	Neurologic diagnosis	Localization of principal brain lesion	Highest serum sodium recorded (mEq./L.)	Highest plasma chlorides recorded (mEq./L.)
Allott, 1939	2	Ruptured aneurysm of anterior cerebral artery	Both frontal lobes	153	119.6
	3	Cerebral infarct	Right internal capsule	162.5	107.5
Sweet *et al.*, 1948	1	Prefrontal lobotomy	Both frontal lobes	171.9	129
	2	Glioma of the third ventricle	Region of the third ventricle	—	129.3
Peters, 1948		Craniopharyngioma	Hypothalamus and infundibulum?	171	—
Biemond, 1949	1	Damage in the region of tuber cinereum	Hypothalamus	155	—
Higgins *et al.*, 1951	1	Cerebral contusion	Base of frontal lobes, etc.	139	111
	2	Cerebral contusion	Base of frontal lobes, etc.	155	123
	3	Cerebral contusion	Base of frontal lobes, etc.	146	118
	4	Cerebral contusion	Base of frontal lobes, etc.	154	115
	5	Cerebral contusion	Base of frontal lobes, etc.	156	107
Cooper & MacCarty, 1951	1	Pituitary adenoma and bilateral occlusion of anterior cerebral arteries	Both frontal lobes and hypothalamus	170	134
	2	Pituitary adenoma	Pituitary.(Hypothalamus?)	162	128
	3	Cerebrovascular accident	Left basal ganglia	161	129
Cooper & Crevier, 1952	1	Cerebral maldevelopment	Diffuse involvement?	160	127
	2	Bilateral subdural hygroma	Diffuse involvement	155	122
	3	Tumor in third ventricle of brain	Hypothalamus	169	132
	4	Craniopharyngioma	Frontal lobes and hypothalamus	152	130

sociated with cerebral lesions (5). Case 1 in that report was studied extensively during a forty-day period from the time of craniotomy for a pituitary adenoma until death. We were impressed with the similarity of the clinical course and laboratory data in this case and in Case 1 in the report of Sweet *et al.* Moreover, it is worthy of note that in our first case there was bilateral occlusion of the anterior cerebral arteries, and one of Allott's patients died following spontaneous rupture of an aneurysm of one of the anterior cerebral arteries. In this regard, it is interesting that McLardy (6) has postulated that the deaths in a series of cases of ligation of the anterior cerebral arteries reported by Dandy (7) may have been due to metabolic abnormalities secondary to the cerebral damage.

Higgins *et al.* (8) have recently reported 5 patients with severe head injury in whom there developed moderately elevated levels of either sodium or chloride, or both. Peters (9) has mentioned a case of extreme sodium retention during the postoperative course of a patient following surgery for a craniopharyngioma.

The independent observation by several investigators of similar unusual electrolyte disturbances associated with neurologic disease, each investigator being impressed with the possibility that the marked retention of sodium and chloride might have been secondary to a cerebral lesion, indicates that this syndrome occurs frequently enough, and is of sufficient significance, to warrant further investigation. Accordingly, we should like to report 4 additional cases in which similar electrolyte disturbances occurred in association with an intracranial disease. The data in each of these cases are incomplete but, nevertheless, believed to be worthy of reporting in view of the basic physiologic implications of the occurrence of unusually marked hypernatremia and/or hyperchloremia associated with lesions of the brain. Only positive data are reported.

CASE REPORTS

Case 1. A 9-month-old male child was hospitalized because of failure of mental and physical development since birth. He appeared malnourished and underdeveloped, weighing only 11 pounds, and was constantly sluggish and drowsy. No focal neurologic abnormalities were present, but the somatic musculature was noted to be unusually flaccid. An electroencephalogram revealed diffuse bilateral abnormal activity. A pneumoencephalogram was attempted but was unsuccessful and permission could not be obtained to repeat this test. Examination of the spinal fluid revealed a chloride level of 152/mEq./liter (normal value 125 mEq./liter). Therefore, serum sodium and plasma chloride levels were determined and found to be 160 and 127 mEq./liter, respectively (normal values: 131–141 mEq./liter and 96–103 mEq./liter, respectively). The hematocrit at this time was 46. The urine had a specific gravity of 1.009 and was acid in reaction. During the ensuing week the serum sodium was determined on 2 occasions and was 155 and 158 mEq./liter, while the plasma chlorides were 122 and 124 mEq./liter on these occasions. Fluid intake during this period varied from 400 to 900 cc. and there was no clinical evidence of dehydration.

Case 2. A 3-month-old female child was admitted to our service because of lethargy, vomiting and progressive enlargement of the head since birth. An electroencephalogram demonstrated diffuse abnormal high voltage slow waves bilaterally. Subdural taps revealed the presence of an accumulation of clear colorless fluid in each subdural space, confirming a diagnosis of bilateral subdural hygroma. The concentrations of serum electrolytes were checked during the preoperative study and serum sodium was 155 mEq./liter, potassium 3.8 mEq./liter (normal value 4–5 mEq./liter) and plasma chlorides, 122 mEq./liter. The carbon dioxide combining power of the blood was 24 mEq./liter. The value for packed red cell volume was 43 per cent. These studies were repeated forty-eight hours later and the values were as follows: serum sodium 148 mEq./liter, serum potassium 3.7 mEq./liter, plasma chlorides 118 mEq./liter and carbon dioxide combining power 27 mEq./liter. This child underwent bilateral trephine drainage of the subdural hygromas, with drainage of the subdural spaces for forty-eight hours postoperatively. During the ensuing week, the blood electrolyte levels were checked on 3 occasions, and values for sodium, potassium, chloride and carbon dioxide combining power were within normal limits. The child was discharged ten days postoperatively.

Case 3. A 58-year-old housewife was admitted to another hospital, three years before admission to our service, because of severe headaches and progressive loss of vision. At that time, a diagnosis of brain tumor involving the third ventricle was made and a ventriculo-cisternal shunt (Torkildsen operation) was performed as a palliative measure. She was symptomatically improved until one month before admission, at which time, she became confused and disoriented, and again complained of decreasing vision. By the time of admission to our service, she was markedly confused, agitated, and completely blind.

The results of neurologic examination were negative except for complete amaurosis and primary optic atrophy with superimposed papilledema. A ventriculogram revealed massive dilatation of both lateral ventricles and the anterior portion of the third ventricle. A round filling defect was seen in the posterior portion of the third ventricle. The Torkildsen catheter was demonstrated to be obstructed. Accordingly, a suboccipital craniotomy and second ventriculo-cisternostomy was performed. However, this procedure was unsuccessful. Consequently, eight weeks after admission, a right frontal craniotomy was performed, and a transventricular removal of a large, brownish vascular tumor was accomplished. The histologic diagnosis was fibroblastic meningioma. The postoperative course was marked by hypotension (B.P. 60/40), hyperthermia (T 104–105° F.) and coma. Six hours postoperatively, respirations became labored and the patient became cyanotic. Respirations ceased on the seventh postoperative day. Urinary output remained adequate (900–1,600 cc. daily) up to the time of death. Permission for necropsy was refused.

At this point, we should like to revert to the day of admission. This patient was admitted with a diagnosis of tumor of the third ventricle, and because of our interest in electrolyte abnormalities associated with lesions in this part of the brain, the levels of serum sodium and potassium, plasma chlorides, and carbon dioxide combining power of the blood were obtained three times weekly from the day of admission. From the time of admission, on August 1, 1951, to the time of the last operation on September 25, 1951, the levels of these electrolytes were within normal limits. However, during the week following the last operation, the level of serum sodium rose to 169 mEq./liter while that of the plasma chlorides rose to 132 mEq./liter. During this period, the fluid intake was maintained by Levine tube feedings at 2,500 cc. per day. Representative values for serum sodium and potassium, plasma chlorides and carbon dioxide combining power of

the blood during the first eight weeks of hospitalization and during the week following removal of the third ventricle tumor are summarized in Table 2. The urine was dilute (specific gravity 1.004) and acid in reaction during the week prior to death. The urinary excretion of chlorides was determined two days before death and was 80 mEq./liter at that time.

TABLE 2. REPRESENTATIVE ELECTROLYTE VALUES IN CASE 3 DURING NINE WEEKS OF HOSPITALIZATION. DOUBLE VERTICAL LINE REPRESENTS TIME OF SURGICAL REMOVAL OF THIRD VENTRICLE TUMOR

	Time (weeks after admission)									
	1	2	3	4	5	6	7	8	8.5	9
Serum sodium, mEq./L.	141	138	135	135	135	133	133	137	164	169
Plasma chlorides, mEq./L.	98	94	98	100	90	92.4	90	101	119	132
Serum potassium, mEq./L.	3.8	4.6	3.8	4.2	4.2	4.6	4	3.8	4.5	4.3
Carbon dioxide combining power, mEq./L.	30	25	27	24	24	22	19	24	30	34

Case 4. A 56-year-old colored female underwent craniotomy and subtotal removal of a craniopharyngioma on June 22, 1951. At the time of surgery, the neoplasm was seen to involve the optic chiasm, anterior cerebral arteries and floor of the third ventricle of the brain. Her postoperative course was uneventful but during the ensuing five months, she became progressively lethargic and died November 17, 1951. Blood and urine electrolyte studies were obtained throughout this five-month period.

No electrolyte abnormalities were noted until August 14, two months postoperatively, at which time the level of plasma chlorides became elevated to 118 mEq./liter. During the next two months, while the patient ate a regular hospital diet containing approximately 150 mEq. of chlorides, the plasma chlorides varied from 103 to 130 mEq./liter. The urine output varied from 600 to 1,600 cc. in twenty-four hours, and contained from 10 to 59 mEq. of chloride per liter. The urine was consistently alkaline, with a specific gravity varying from 1,005 to 1,024. From October 26 through November 4, the patient received a salt-free diet. However, the level of plasma chlorides remained elevated and varied from 112 to 125 mEq./liter. Paradoxically, during this period of sodium chloride-free diet, the urinary excretion of chlorides increased, and varied from 47 mEq./liter on October 29 to 122 mEq./liter on November 1. Urinary output on these dates was 900 and 950 cc. per twenty-four hours, respectively. No appreciable change was observed in the blood electrolytes when salt was again added to the diet during the last two weeks of life. The concentration of serum sodium varied from 139 to 152 mEq./liter during the five months of observation.

There was no evidence of dehydration during the five months of observation. The blood nonprotein nitrogen level did not rise above 32 mg. per cent, while the hematocrit

averaged 41 per cent. The urea clearance test was performed twice during November and the results were found to be within normal limits. The level of plasma proteins on November 2 was 6.0 grams per cent.

During the last month of life, there was a marked edema of the left extremities and left side of the face. This remained localized to the left limbs and face until one week prior to death, when the right limbs became slightly edematous. All four limbs became extremely flaccid and the deep tendon reflexes could not be obtained, during the last two months of life. During this time, the patient became progressively more drowsy and stuporous and she died on November 17, 1951. Permission for necropsy was denied. Representative laboratory data from this case are summarized in Table 3.

TABLE 3. REPRESENTATIVE LABORATORY DATA FROM CASE 4

	Date						
	July 15	Aug. 15	Sept. 24	Oct. 15	Oct. 23	Nov. 1*	Nov. 15
Serum sodium, mEq./L.	144	148	147	152	143	143	146
Plasma chlorides, mEq./L.	107	118	127	130	125	125	115
Serum potassium, mEq./L.	4.1	4.0	4.1	4.0	3.8	3.7	3.4
Carbon dioxide combining power, mEq./L.	27	26	23	22	19	21	23
Urinary chlorides, mEq./L.	59	28	28	13	58	122	48

* Following six days of salt-free diet.

DISCUSSION

The pathogenesis of the unusually marked elevation of serum sodium and plasma chlorides in the foregoing cases, and in the cases cited in the literature, is not readily explained. Sufficient data are available in each of the 4 cases reported here to rule out hypertonicity of the blood due to dehydration. There was no evidence of insufficient fluid intake or excess water loss in any of these patients. Moreover, Allott (1) has previously pointed out that most patients with severe dehydration do not demonstrate such elevated levels of these ions in the blood. There was no evidence of renal disease in these cases, and it is unlikely that any morphologic changes in the kidneys could have been responsible for the electrolyte abnormalities (10, 11).

Although hyperfunction of the adrenal cortex can produce hypernatremia, the level of serum sodium in such instances seldom exceeds 150 mEq./liter (12, 13). Sweet *et al.* (2) have suggested that the hypernatremia and hyperchloremia associated with some cerebral lesions might be due to an exaggerated alarm reaction. However, the mechanism of electrolyte retention in the alarm reaction and its relation, if any, to the adrenal cortex is not clear.

Allott (1), Sweet *et al.* (2), Higgins *et al.* (8), and Cooper and MacCarty (5) have all suggested that cerebral lesions may play an important role in the development of unusually marked hypernatremia and hyperchloremia. Allott postulated that varied intracranial lesions might in some way act through interference with the secretion of the antidiuretic hormone, thus producing excessive loss of water and causing retention of chlorides due to the absence of the chloruretic effect of this hormone. All of these investigators have reported a markedly decreased urinary excretion of sodium chloride in their cases. Therefore, the possibility that some abnormality in the renal tubular reabsorption of these ions existed in these cases must be considered. Allott has stated that "the true explanation seems to be that the tubules handle chloride and sodium in a similar manner to glucose, and reabsorb up to a certain maximum quantity per unit time, whatever the level in the blood."

It is our opinion that the hypernatremia, hyperchloremia and hypochloruria in these cases are secondary to the cerebral lesions. The frequency of such markedly elevated levels of sodium and chloride in the blood associated with cerebral disorders seems to us to suggest a causal relationship. This hypothesis receives considerable support from several physiologic investigations. Lewy and Gassmann (14) reported the occurrence of elevated levels of chloride in the blood of cats following the production of lesions in the paroptic nucleus of the hypothalamus, and this nucleus has been demonstrated to be functionally related to the pars nervosa of the pituitary gland (15). Stevenson, Welt and Orloff (16) have recently reported that the experimental production of lesions in the ventro-median nuclei of the hypothalamus of rats resulted in an abnormal elevation of sodium in the serum. This increase is probably of a chronic nature, having been observed by them three to four months after placement of the lesions. These authors believe it is unlikely that the adrenal cortex plays a predominant role in causing the abnormalities shown by the hypothalamic animals. They believe that the rats are in a chronic state of relative dehydration and may suffer "hypothalamic hypodipsia." However, in one case of marked hypernatremia and hyperchloremia associated with a cerebral lesion, superhydration was carried out to the point of water intoxication without lessening the hypertonicity of the blood (17).

The presence of an "osmo receptor" which responds to the effective osmotic pressure of the blood serum has been suggested by Verney (18, 19). He further states that the neurohypophysis responds to the osmotic pressure of the blood by regulating the secretion of antidiuretic hormone proportionately to changes in the concentration of osmotic components of the serum, 90 per cent of which consist of sodium salts. We believe that the high concentration of sodium and chloride in the blood in these cases is caused by interference with the sensitivity of, or damage to, the cerebral osmo receptor mechanism of Verney. We do not want to compound this hypothesis with the additional speculation of a specific cerebral structure in which the osmo receptor can be localized. However, it is worthy of note that the frontal lobes and/or hypothalamus were principally involved in 16 of the 18 cases summarized in Table 1. Moreover, Sweet *et al.* (2) have pointed out the strong possibility "that a lesion of the structures around the third ventricle or of the posterior part of one frontal lobe may cause hyperchloremia and azotemia." The studies of Stevenson *et al.* (16) and Lewy and Gassmann (14) are also relevant to this consideration, inasmuch as the former workers have produced hypernatremia in rats by experimental hypothalamic lesions, and the latter investigators have reported hyperchloremia following hypothalamic lesions in cats.

If these electrolyte abnormalities are, indeed, secondary to the cerebral lesions in these cases, one might expect to encounter occasionally an opposite clinical picture of sodium or chloride depletion associated with disease of the brain. Such a syndrome has, in fact, recently been described by Hilden (20) who reported 5 cases of encephalopathy associated with acute cerebral disturbances, hypertension and a fall in the level of plasma chlorides.

Further speculation is beyond the scope of this report. However, the physiologic implications of bizarre neurogenic electrolyte abnormalities are far reaching and deserve further investigation. The practical implications of these unusual electrolyte findings in cases of cerebral lesions are quite evident. The high mortality rate following neurosurgical procedures in the region of the third ventricle and hypothalamus has always been attributed to the effect on "vital centers" located in the diencephalon. The precise cause for postoperative death in many cases, however, often remains obscure. Many neurosurgeons have been impressed with the sudden respiratory failure which often follows removal of a tumor from the region of the hypothalamus. In view of the fact that experimental hypertonicity of extracellular fluid, due to excess sodium and chlorides, results in death from depression of the nervous system and respiratory failure (21, 22), one wonders whether the extreme hypertonicity in such instances as that

cited in Case 3 of this report, may not contribute to the death of some patients following intracranial surgery.

SUMMARY

Fourteen cases of marked hypernatremia and/or hyperchloremia associated with cerebral lesions previously reported in the medical literature have been reviewed, and 4 additional cases are presented. The cerebral lesions associated with these disturbances usually involve the frontal lobes or hypothalamus. We believe that the clinical and experimental evidence at hand indicates these unusual electrolyte abnormalities to be secondary to the cerebral disorders. Accordingly, the markedly elevated levels of serum sodium and plasma chlorides in these cases are considered to be neurogenic. The mechanism whereby cerebral lesions may cause retention of these electrolytes is not clear. It is suggested that damage to a cerebral osmo receptor mechanism may be involved in the production of this syndrome.

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NEUTRAL STEROID EXCRETION BY NORMAL AND BY SCHIZOPHRENIC MEN*

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IN PREVIOUS papers the demonstration of abnormal adrenocortical response to stress and ACTH by schizophrenic men has been made (1, 2, 3). As possible explanations of the apparent diminished responsiveness in a large proportion of the schizophrenic subjects we have suggested: a) a tendency for the rapid inactivation of either endogenous or exogenous ACTH in schizophrenics or b) limited steroidogenesis by the schizophrenics' adrenal cortices in response to increased circulating ACTH from either exogenous or endogenous sources. Although excessive ACTH inactivation cannot be overlooked, certain considerations suggest that abnormal steroidogenesis in response to ACTH may occur in schizophrenic men. The fact that the total basal 17-ketosteroid output of schizophrenic men equals or exceeds that of nonpsychotic men of the same age, whereas the basal corticosteroid output (measured as neutral reducing lipid) is significantly lower in schizophrenic men, suggests a differential rate of secretion of the adrenal precursors of these two classes of urinary steroids. As an approach to the nature of steroidogenesis by schizophrenic men, we have undertaken the analysis of certain neutral urinary steroids in schizophrenic and control subjects. We have measured in urine: a) the total 17-ketosteroids by the application of the Zimmermann color reaction, b) the 17-ketosteroids chromogenic to $SbCl_3$, c) the α and β-ketosteroids by the Zimmermann reaction, and d) the nonketonic steroids chromogenic to $SbCl_3$.

METHODS AND SUBJECTS

Urines were extracted and fractionated into neutral ketonic and nonketonic fractions by methods previously described (4). Corticosteroids were extracted and measured as neutral reducing lipid in the total neutral fraction (5) by the Heard-Sobel method (6). The antimony trichloride ($SbCl_3$) reaction (7) was applied to both the neutral ketonic and nonketonic

Received for publication January 19, 1952.

* The investigations described in this paper were aided by grants from the George I. Alden Trust and the U. S. Public Health Service (CG-999).

fractions of the hydrolyzed urine. The standard chromogen used was androsterone, and $SbCl_3$ reaction data are presented as androsterone equivalents. It is obvious that the nonketonic fractions do not contain chromogenic ketone but rather, nonketonic chromogen (8); nonetheless, the data for nonketonic neutral lipid are, for convenience, expressed in androsterone equivalents. The ketonic neutral extracts of acid-hydrolyzed urines were separated into α and β fractions with digitonin, as previously described (4), but cholesterol was added as the co-precipitant instead of dehydroisoandrosterone. Cholesterol has the advantage of not reacting with *m*-dinitrobenzene, the color reagent used for measurement of the ketones of the β fraction. One hundred micrograms of cholesterol were added per 0.5 mg. of total neutral 17-ketosteroid.

For the study of the excretion of neutral ketones and neutral reducing lipid three groups of subjects were employed (Table 1), namely, one group of 21 schizophrenic men and two groups of nonschizophrenic men, one

TABLE 1. THE EXCRETION OF NEUTRAL KETONIC STEROIDS IN THREE GROUPS OF SUBJECTS. (OUTPUT AS MG./GM. CREATININE.)

Group	Number of subjects	Mean age (yrs.)	I Total 17-KS	II Beta 17-KS	III Beta as % of total 17-KS	IV $SbCl_3$ chromogen	V $SbCl_3$ as % of total 17-KS
Younger normal subjects	13	29.0	8.38 ±0.79	0.499* ±0.062	6.39* ±0.87	5.90 ±0.09	62.8 ±4.5
Older normal subjects	14	54.2	4.70 ±0.31	0.274 ±0.031	5.88 ±0.57	2.52 ±0.17	53.7 ±2.6
Schizophrenic patients	21	34.6	7.10 ±0.68	0.838 ±0.134	11.43 ±1.06	3.94 ±0.31	61.3 ±2.5

* Underlined values are significantly different from the corresponding values for the schizophrenic patients: ——— = $P < 0.01$; . . . . = $P < 0.05$ in the *t* test.

consisting of 14 older subjects (aged 40 to 59 years) and the other of 13 younger subjects (aged 20 to 39 years). In the study of nonketonic steroids (Table 2) data were obtained on 19 schizophrenic subjects (aged 25 to 43) not identical with those contributing to the ketosteroid study, and on two groups of control subjects: a) the 13 younger men previously mentioned, and b) arthritic men (aged 25 to 64).

The control output specimens were taken under fasting conditions, *i.e.*, in the morning following a minimum fast of eleven hours. At the conclusion of this control urine collection, each subject was given a standard dose of 25 I.U. of ACTH and urine was again collected over a period of from three and a half to four hours following ACTH injection. Creatinine outputs were determined for each urine specimen (*cf.* 1).

TABLE 2. THE MEAN EXCRETION OF NEUTRAL NONKETONIC STEROIDS BY THREE GROUPS OF SUBJECTS. (OUTPUT AS MG./GM. CREATININE.)

Group	Number of subjects	Mean age (yrs.)	Nonketonic steroid output
Younger normal subjects	13	29.0	$\underline{6.72 \pm 1.32}$*
Arthritics	18	47.8	$\underline{6.81 \pm 1.15}$
Schizophrenic patients	19	34.0	2.47 ± 0.213

* Underlined values are significantly different from the corresponding values for the schizophrenic patients: ——— = $P < 0.01$.

RESULTS

The control output of urinary steroids

In Table 1 are the mean data for the basal output of 17-ketosteroids in the three groups of subjects. A significantly lower output of total 17-ketosteroid, measured either as *m*-dinitrobenzene (column I) or $SbCl_3$ (column IV) chromogen, occurs in the data for the older subjects. This age difference has been previously noted (9, 10). When these data for the schizophrenic subjects are compared with the corresponding data for the younger men of comparable age, no significant quantitative difference is observed. Furthermore, the $SbCl_3$ chromogen output taken as a percentage of the *m*-dinitrobenzene chromogen (column V) is not significantly different in the three groups, although a somewhat lower value is noted for the older subjects.

Examining the data for β-ketosteroid output (column II) we find characteristic output levels for each of the three groups, the schizophrenics excreting the largest amount—approximately 75 per cent more than the younger control subjects and 220 per cent more than the older subjects. When the β-ketosteroid is taken as a percentage of the total (column III) the values for the older and younger control subjects do not differ significantly, whereas the value for the schizophrenic subjects is significantly higher than that for each control group by 80 to 95 per cent.

Since the percentage of β-ketosteroid does not differ significantly in the

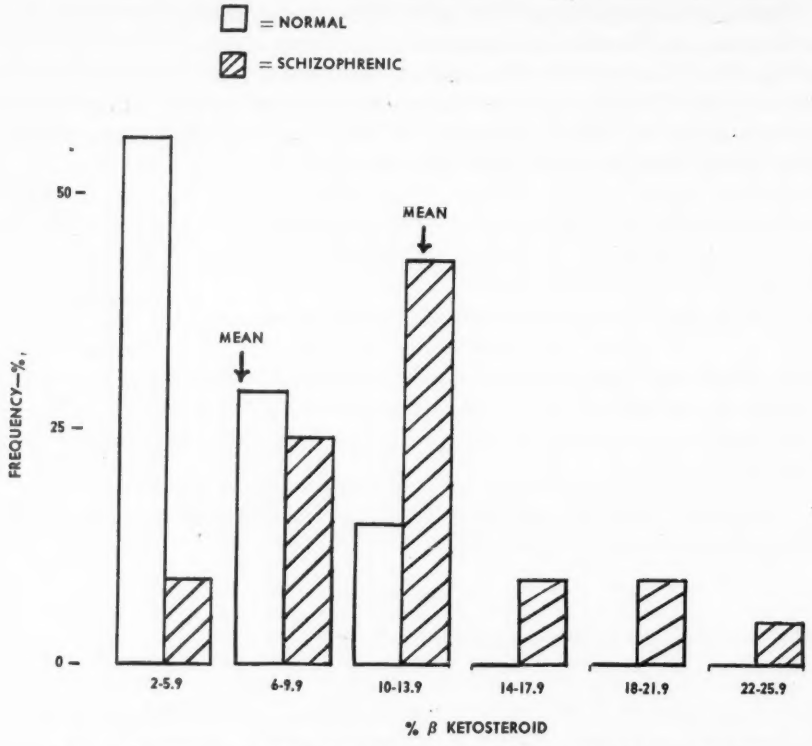

FIG. 1. Frequency distribution of percentage of β-ketosteroid excretion for control and schizophrenic subjects.

younger and older control groups, we have compared the frequency distribution among the control subjects with that of the schizophrenic subjects. These are plotted in Figure 1. It is clear that there is a certain amount of overlapping, but the difference between the two sets of data is obvious.

The β-ketosteroids are considered as fairly direct indices of rather specific adrenal precursors (11) and their output has been considered diagnostic for certain types of steroid-producing adrenal tumors (12). The neutral reducing lipids may be taken as a measure of urinary corticosteroid (5). Accordingly, the data of those subjects for whom we have measurements of both (14 of the normals and 13 of the schizophrenic subjects of Table 1) have been compared, and significant positive correlations have been found (Fig. 2). When these subjects are divided into three groups, *viz.*, low, intermediate and high excreters, and the percentage of β-ketosteroid is compared with the neutral reducing lipid output, it is noteworthy that the slope of the regression line for the schizophrenics' data is approximately

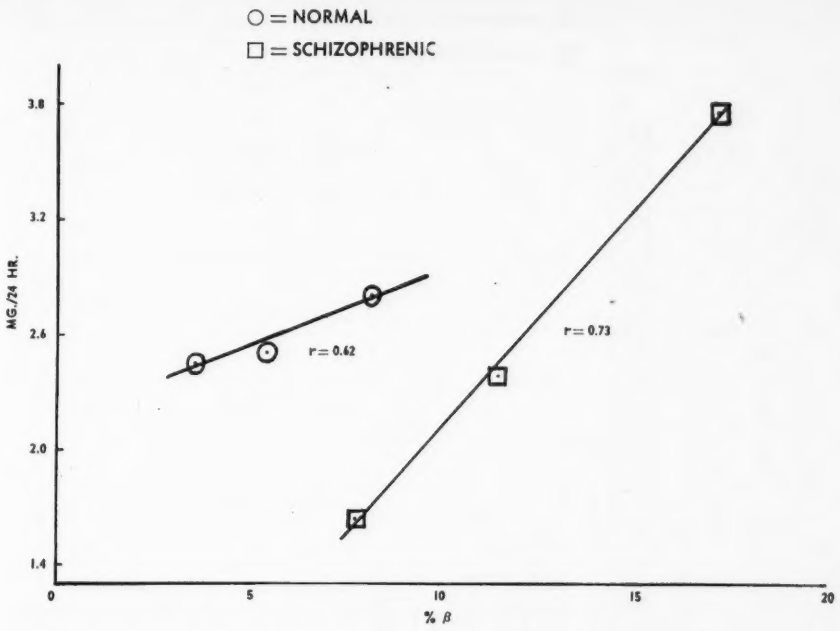

FIG. 2. Relationship of neutral reducing lipid to percentage of β-ketosteroid excretion.

three times as steep as that for the normals' data. This suggests that for every increment of increase in neutral reducing lipid output there is an increment of output of β-ketosteroid which is threefold greater in the schizophrenics than in the normal subjects.

The control outputs of nonketonic steroids for three groups of subjects are presented in Table 2. There is a highly significant lowered output in the schizophrenic subjects, but no significant difference in output between the two nonschizophrenic groups. Grouping together the data for the latter, and comparing the frequency distribution with that for the schizophrenic subjects, we arrive at the distributions shown in Figure 3. The limitation of the schizophrenics' output values to the two lowest frequency classes, with approximately 80 per cent of the values falling within the the range 0–2.9 mg. per gram of creatinine, is in marked contrast to the greater scatter and range of the data for the control subjects.

The effects of ACTH

Most of the subjects contributing the foregoing data received 25 I.U. of ACTH as described. The urinary outputs of the neutral ketonic steroids following ACTH are presented in Table 3.

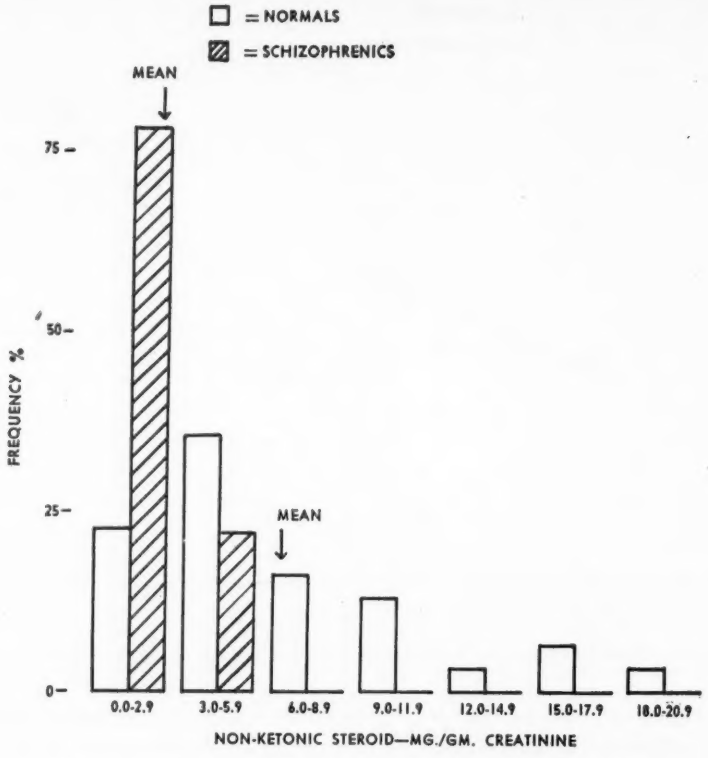

FIG. 3. Frequency distribution of nonketonic steroid excretion for control and schizophrenic subjects.

The data in Table 3 are similar to those demonstrated in the pre-ACTH period (Table 1): a) the total 17-ketosteroid output (although elevated as the result of ACTH administration) is not significantly different in the control and in the schizophrenic groups of similar age (column I), b) the absolute ouput of β-ketosteroid (column II) is again higher in the schizophrenic subjects, c) the β-ketosteroid as a percentage of the total (column III) is 240 per cent higher in the schizophrenic group, and d) the $SbCl_3$ chromogen (column IV) in the schizophrenic subjects is significantly lower than is the corresponding output for the younger control group, but significantly higher than the older group's value. The percentage output of $SbCl_3$ chromogen (column V) in the younger control subjects significantly exceeds that of the schizophrenics, and also that of the older control group.

Comparing the data of Table 1 and Table 3, one observes with ACTH an increased output of all types of ketonic steroids measured in all groups

TABLE 3. THE EXCRETION OF NEUTRAL KETONIC STEROIDS IN THREE GROUPS OF SUBJECTS FOLLOWING ACTH ADMINISTRATION. (OUTPUT AS MG./CM. CREATININE.)

Group	Number of subjects	Mean age (yrs.)	I Total 17-KS	II Beta 17-KS	III Beta as % of total 17-KS	IV $SbCl_3$ chromogen	V $SbCl_3$ as % of total 17-KS
Younger normal subjects	13	29.0	11.47 ±0.85	0.552* ±0.067	4.76* ±0.31	9.72 ±0.86	81.1 ±4.3
Older normal subjects	14	54.2	5.88 ±0.46	0.139 ±0.039	5.46 ±0.55	3.05 ±0.30	51.5 ±2.8
Schizophrenic patients	21	34.6	9.17 ±0.86	0.910 ±0.161	11.34 ±1.35	4.99 ±0.48	56.8 ±2.7

* Underlined values are significantly different from the corresponding values for the schizophrenic patients: ——— = $P < 0.01$; ..... = $P < 0.05$ in the t test.

of subjects, but the schizophrenics' output is increased less than that of the younger control group in each instance. Thus a 37 per cent increase in the output of total 17-ketosteroid occurs among the younger controls, whereas the schizophrenics' output increases 29 per cent; the corresponding figures for β-ketosteroids are 11 per cent and 8.5 per cent; and for $SbCl_3$ chromogen, 65 per cent and 26.5 per cent, respectively. A diminished responsivness to ACTH in terms of 17-ketosteroid output has been previously noted (1, 2, 3).

TABLE 4. THE MEAN EXCRETION OF NEUTRAL NONKETONIC STEROIDS BY THREE GROUPS OF SUBJECTS FOLLOWING ACTH ADMINISTRATION. (OUTPUT AS MG./CM. CREATININE.)

Group	Number of subjects	Mean age (yrs.)	Nonketonic steroid output
Younger normal subjects	13	29.0	4.89 ±1.14
Arthritics	14	46.5	4.61 ±0.642*
Schizophrenic patients	19	34.0	2.62 ±0.334

* Underlined values are significantly different from the corresponding values for the schizophrenic patients: ——— = $P < 0.01$.

The nonketonic steroid output following ACTH administration is presented in Table 4. The schizophrenics' output is less than that of the control groups, but is significantly so in only one instance. This is due to the fact that, following ACTH, the nonketonic steroid output declines in the control subjects (by about 30 per cent) but increases slightly in the schizophrenics.

DISCUSSION

The foregoing data demonstrate that the neutral steroid output of schizophrenic subjects is in some respects similar to, and in others significantly different from, the outputs of control subjects. This difference persists after a single adrenal-stimulating injection of ACTH. There are clear quantitative differences in basal β-ketosteroid and nonketonic steroid outputs, but in the schizophrenics the former is greater whereas the latter is less. The suggestion of a steroid dysgenesis is emphasized by such data, since increased output of certain steroids and decreased output of others cannot be ascribed to a simple quantitative alteration in steroidogenesis. Whether or not the observed differences in urinary steroid excretion reflect differences in the nature of the adrenal secretory product is more difficult to ascertain from our data. It is possible to deduce that processes in the intermediary metabolism of ketosteroid and nonketonic steroid precursors differ to the extent that nonschizophrenic subjects tend to produce less β-ketosteroid and more nonketonic steroid than do schizophrenic patients. The nature and locus of such processes are difficult to envisage. An explanation of these data in terms of altered adrenal steroidogenesis is more inviting. On this basis, schizophrenic subjects tend to produce a larger proportion of adrenal β-ketosteroid precursors but a lower proportion of nonketonic steroid precursor. Under the influence of ACTH, the β-ketosteroid precursors increase in both schizophrenic and normal subjects but the nonketonic steroid precursors decline in the normal subjects. Perhaps ACTH-induced steroidogenesis in the normal subjects causes a relative shift to ketosteroid precursors, whereas this does not occur in schizophrenic men. Clearly, further study is required to elucidate these possibilities. We propose undertaking an analysis of specific urinary substances and also a direct study of adrenal steroidogenesis in adrenal glands taken from schizophrenic and from nonschizophrenic sujects.

SUMMARY

The basal urinary output of total 17-ketosteroid, β-ketosteroid and $SbCl_3$ chromogenic ketosteroid has been studied in 21 schizophrenic men and in 27 nonschizophrenic (13 younger and 14 older) subjects. The older subjects excreted, on the average, less of each steroid type than did the

other groups of subjects. This appears to be in part a function of age, since the younger control subjects did not excrete amounts of total 17-ketosteroid significantly different from those of the schizophrenics. The β-ketosteroid excretion of the schizophrenic men significantly exceeded that of both control groups. The β-ketosteroid output, taken as a percentage of the total 17-ketosteroid, was similar in the younger and in the older groups of control subjects, but was significantly elevated above the control values in the schizophrenics' data. The $SbCl_3$ chromogen, taken as a percentage of the total 17-ketosteroid was not notably different in the three groups of subjects.

Basal urinary nonketonic neutral steroid measured as $SbCl_3$ chromogen has been determined for the 13 younger control subjects, for a group of 18 arthritic men, and a for a group of 19 schizophrenic men. There was no significant output difference between the two nonschizophrenic groups, but the schizophrenic subjects, on the average, showed a highly significant lower output than did the controls.

The administration of 25 I.U. of ACTH increased, in a $3\frac{1}{2}$-hour postinjection period, all types of ketosteroid output in all groups of subjects. The increases shown by the schizophrenic subjects tended to be less than those of the control group of similar age, yet a significantly higher β-ketosteroid output by the schizophrenic subjects persisted. The output of $SbCl_3$ chromogen in this younger control group was significantly higher than in the schizophrenic subjects. Nonketonic neutral steroid excretion declined markedly after ACTH in the control subjects, but increased slightly, on the average, in the schizophrenic subjects; despite this, the schizophrenics' output was still markedly less than that of the control subjects.

It is suggested that the differences in steroid excretion between control and schizophrenic subjects may be inherent in a steroid dysgenesis by schizophrenics.

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THERAPEUTIC STUDIES IN HYPERTHYROIDISM: METHIMAZOLE

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INTRODUCTION

In the last few years many new agents have been presented to the clinician for the treatment of hyperthyroidism. A great deal of experience and the collected reports of many investigators are necessary before the final role of each of these substances can be definitely determined. The burden of this report will be to contribute experience with one of these new agents, methimazole.[1]

Methimazole (1-methyl-2-mercaptoimidazole) was first reported by Stanley and Astwood (1) as "an antithyroid compound highly effective in man" in June, 1949. They observed that as small a dose as 0.5 mg. had a marked inhibitory effect on the uptake of radioactive iodine by the thyroid gland. Five milligrams completely inhibited the uptake for twenty-four hours.

In 1950 two papers appeared to further the use of the drug. Beierwaltes (2) treated 9 patients of various ages; 6 showed a rapid return to a normal basal metabolic rate, but 3 did not show any return to a normal rate over a period of three months or more. He estimated the optimum dose to be 40 mg. per day and indicated that he had observed no toxic reactions. Reveno and Rosenbaum (3) treated 18 patients and reported no toxic reactions. They estimated the optimum dose to be from 2 to 5 mg. every eight hours. Lahey (4), on the other hand, has reported the incidence of reactions from methimazole to be 3 per cent. Bartels (5) later reported an incidence of 6 per cent for skin reactions, of which 3 per cent were serious enough to require discontinuance of therapy. He observed 1 case of severe granulocytopenia.

Received for publication December 17, 1951.

* The authors wish to acknowledge the assistance of Doctors B. Catz, R. Stirrett, B. Simkin, F. Scharles and C. Sturgeon, members of the Thyroid Clinic; and the technical assistance of Miss Shirley Templin and Mr. Salyer Reed.

[1] Methimazole (Tapazole®) was furnished through the courtesy of Dr. D. C. Hines of the Eli Lilly & Co., Indianapolis, Indiana.

PURPOSE AND METHODS

Two specific questions were posed when this study was undertaken. First, how effective is methimazole as an antithyroid drug? Secondly, how toxic is the drug in clinical use?

Cases of hyperthyroidism were selected from the thyroid clinic at the Los Angeles County General Hospital. Patients were seen at weekly or biweekly intervals as each specific situation indicated. Some patients were of necessity hospitalized, but in each instance the clinic staff continued general direction of the case.

The ages of the patients ranged from 21 to 72 years; 24 were females and 5 were males. The group was heterogeneous and characteristic of the patients seen in our clinic; no special selection was made.

Previous studies here (6) had demonstrated the effectiveness of the protein-bound iodine test as an index of thyroid activity. The patients studied had repeated protein-bound iodine determinations, basal metabolism tests, serum cholesterol determinations, and blood counts, in addition to meas-

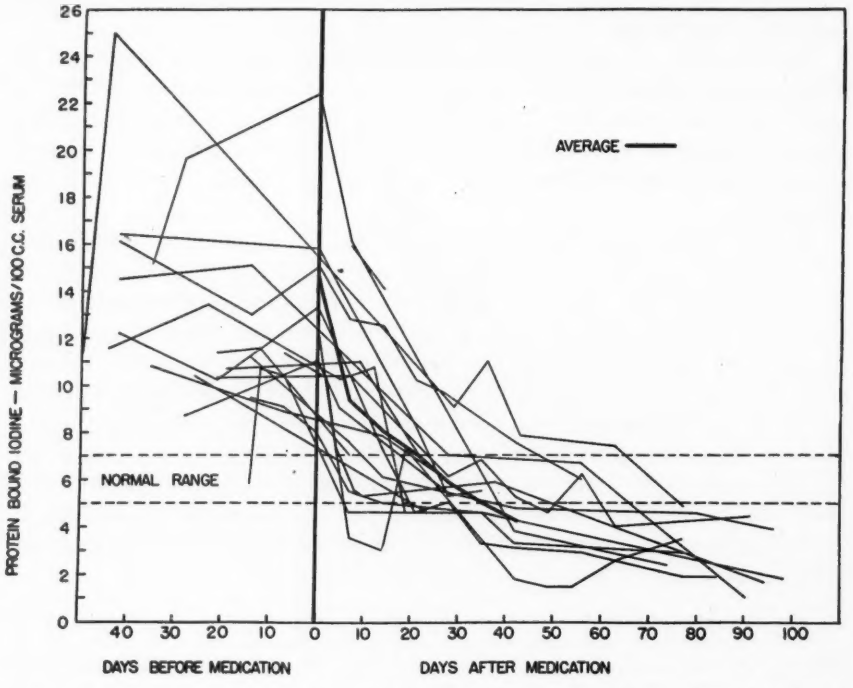

FIG. 1. Response of the protein-bound iodine level in a group of hyperthyroid patients treated with methimazole. The heavy black line represents the average response.

urements of neck size, estimations of gland weight, and ophthalmometry when indicated, both before and during treatment. A number also had radioiodine uptake studies.

The initial dose of methimazole was usually 30 mg. daily, given in three doses of 10 mg each. Four patients were started with 60 mg. per day and 1 with 15 mg. per day.

RESULTS

Methimazole was administered to 29 patients with hyperthyroidism. Five of these, however, took the drug so briefly that no conclusions could be drawn. One of these patients was psychotic, 1 did not return, 1 had local myxedema, 1 refused the medicine after two doses, and 1 received the drug for two weeks only as preparation for radioactive iodine therapy.

Of the 24 patients who continued the medication, 21 had a good therapeutic result. Of the 3 who failed to respond, 2 had severe hematologic reactions. The third case gave an unsatisfactory response.

The average patient reached a euthyroid state within two to four weeks. The slowest to respond required ten weeks, whereas 1 patient became clinically normal in one week (Fig. 1).

Five patients studied had previously received radioactive iodine; but because of the urgency of the disease and because of the slowness of response to radiation, methimazole was administered a few days after the I^{131} in order to relieve the patients more rapidly of their symptoms.

Eight patients who responded favorably to methimazole subsequently received radioactive iodine for more permanent control of the disease.

There was a tendency to relapse once the drug was discontinued. The relapse, when it was observed, occurred from three to four weeks after cessation of the treatment.

Toxicity

Toxic reactions were observed in 8 subjects. The most common disorder was a macular rash which occurred in 4 subjects. One patient had hives, 2 complained of nausea, and 1 had a minor episode of vomiting.

In 2 cases serious reactions developed. One patient responded with leukopenia and fever; the other had thrombocytopenic purpura. Their case reports follow:

Case 1, L. M., was a 31-year-old Negress. Tremor and nervousness developed post partum in 1948. Her symptoms remained mild until July 1950, when she became increasingly nervous, lost weight, and had diarrhea and exophthalmos. On October 31, 1950, she was hospitalized because of fever, chills, and a cough.

Physical examination revealed extreme nervousness and exophthalmos. Her blood pressure was 150/50, pulse 120 and temperature 101° F. The thyroid was diffusely en-

larged and a bruit was heard. The remainder of the examination gave negative results.

The initial white blood cell count was 7,000 with 60 per cent polymorphonuclear leukocytes. The concentration of protein-bound blood iodine was 22.9 micrograms per cent. A study with radioactive iodine showed an uptake of 44 per cent in twenty-four hours.

For the first seven days the temperature remained below 99.8° F. On the eighth day the administration of thiouracil and potassium iodide was begun. Three days later her temperature rose to 101° and the pulse became quite rapid. Two days later she presented a clinical picture of impending thyroid crisis. The possibility of sensitivity to either thiouracil or iodide was considered and the drugs were stopped. Her hyperthyroidism continued.

On November 15, 1950, methimazole was begun in a dose of 60 mg. daily. The fever continued and a leukopenia developed; the cell count was 2,600 with 23 per cent polymorphonuclear leukocytes. On November 23, 1950, methimazole was stopped. This time the temperature fell to 99° F. Three subsequent attempts to control the hyperthyroidism with methimazole resulted each time in a febrile reaction and a leukopenia. On December 21, three days after the fourth trial of methimazole, the lowest white cell count was recorded—1,800 leukocytes of which 48 per cent were polymorphonuclear.

Examination of material obtained by sternal puncture revealed only a "reactive bone marrow."

Following cessation of the drugs the white count returned to normal.

Despite extensive investigation, no cause for the fever and acute illness was found other than hyperthyroidism. Subsequent treatment with radioactive iodine effectively controlled her disease.

Case 2, C. R., a 24-year-old Mexican woman, was first seen December 5, 1950. Her complaints were increasing nervousness, weakness, irregular menses, and a weight loss of ten pounds in three months.

Physical examination revealed tremor and a stare but no measurable exophthalmos. Her pulse was 136. No thyroid enlargement could be demonstrated. The white blood cell count was 8,100 with 53 per cent polymorphonuclear leukocytes; the protein-bound blood iodine was 19.1 micrograms per cent.

She was started on 60 mg. of methimazole daily and her toxicity rapidly abated. On the fifth day of treatment, the white count was reported as 5,800 with 75 per cent polymorphonuclear cells; on the eighth day, 4,500 (64 per cent p.); and on the tenth day, 3,700 (60 per cent p.). Epistaxis developed on that day and the platelet count was found to be 37,000. The next day her white count was 5,000 (23 per cent p.) and the platelet count was 41,000. She had marked epistaxis and bleeding gums.

Methimazole was stopped.

Six days later the platelet count had risen to 120,000 and all signs of bleeding had disappeared. By the twelfth post-treatment day the white count was 6,500 with 60 per cent polymorphonuclear cells and the platelets were entirely normal.

She was subsequently adequately controlled with iodothiouracil (Itrumil®).

CONCLUSIONS

Methimazole is an effective antithyroid drug. In hyperthyroidism therapeutic response is usually achieved in from two to four weeks after institution of therapy.

The drug will probably find its principal use in the preparation of the

hyperthyroid patient for surgery. In this capacity it compares favorably with propylthiouracil. Its toxicity, however, is somewhat higher. Eight of our patients showed some toxicity, although in only 2 of these was it considered serious.

SUMMARY

1. Twenty-nine patients with hyperthyroidism were given methimazole; 5 continued the medication only briefly; 24 persisted to the conclusion of the study.

2. Twenty-one of the 24 had a good therapeutic response.

3. The usual effective amount was 30 mg. per day in divided doses.

4. Toxicity was observed in 8 patients. In 2 of them serious reactions developed—leukopenia in one, and thrombocytopenia in the other.

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THE MENSTRUAL PATTERN IN THYROID DISEASE*†

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INTRODUCTION

CLINICIANS have long recognized that diseases of the thyroid gland in the premenopausal female often are associated with abnormalities of menses. Most texts on the subject describe amenorrhea in patients with thyrotoxicosis, and menorrhagia in patients with myxedema. W. O. Thompson (1) and Lerman (2) emphasized how little is actually known of the relationship of the thyroid and menstruation. It is not clear whether the menstrual dysfunctions occur because of abnormalities of the pituitary, of the end organs involved, or of utilization of the various hormones peripherally. The observations reported here were obtained in order to describe and define further the menstrual abnormalities of thyroid disease.

METHODS

Premenopausal female patients attending the Thyroid Clinic of the Massachusetts General Hospital over a twelve-month period, who showed definite clinical and laboratory evidence of hyperthyroidism or of myxedema, were included in this study. Thyroid status was established by the usual procedures, including determinations of serum precipitable iodine (PBI) (3) and the thyroidal accumulation of I^{131} (4). Every effort was made to ascertain the actual dates of the menstrual flows for the preceding several months. Each patient kept a chart on which she listed the dates on which subsequent flows started and stopped, the presence or absence of cramps at the times of the periods, and the daily morning basal body temperature. Before therapy directed at the thyroid disease was begun, a urine specimen was obtained for pregnanediol assay (5), and when indicated, for measurement of gonadotropins (6); one or more endometrial biopsy specimens were also obtained, using a Meigs curette. Only the results of pregnanediol

Received for publication January 15, 1952.

* Read at the Annual Meeting of the Association for the Study of Internal Secretions, Atlantic City, N. J., June 7–9, 1951.

† This investigation was supported in part by a generous grant from Parke, Davis and Company, Detroit, Michigan.

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assays performed within the fifteen days immediately preceding Day 1 of a menstrual flow are included here. The endometrial biopsy specimens were obtained during the ten-day period preceding Day 1 of a menstrual flow, and occasionally within the first thirty-six hours, if the flow was minimal. Before starting treatment in patients with amenorrhea, usually two endometrial biopsy specimens were taken approximately two weeks apart. We are reporting only those biopsies which have been interpreted in essentially the same way, by one of us (SHS) and by two of the members of the Department of Pathology of the Massachusetts General Hospital.

The results of the biopsies have been graded as follows: *proliferative:* +, indicating endometrium that is typical of the postmenopausal state, in that it is proliferative in type but is inactive, atrophic tissue; *proliferative:* ++, indicating endometrium that is proliferative in type with minimal estrogen effect, such as is seen before Day 9 of a normal 28-day menstrual cycle; *proliferative:* +++, indicating endometrium that is proliferative in type showing normal, active, preovulatory proliferation such as is seen on about Day 12; and *proliferative:* ++++, indicating endometrium that is proliferative in type and demonstrating a degree of hyperplasia not seen during a normal cycle. *Secretory, early,* indicates endometrium of the secretory type such as is seen on Days 15–21 of a normal 28-day cycle; *secretory, mid.,* indicates endometrium of the secretory type such as seen on Days 22–25; *secretory, late,* indicates endometrium of the secretory type as seen on Days 26–28; and *menstrual, postovulatory,* indicates endometrium of the late secretory type with some evidence of breakdown and bleeding. If the type of secretory endometrium was that expected on the day the biopsy specimen was taken, it was called normal. The term *inadequate corpus luteum effect* has been used to indicate that a biopsy showed a secretory effect, but not as advanced as should be found on that day of a standard 28-day cycle, *e.g.,* an early secretory effect two days before the flow.

The term *metropathia hemorrhagica* is used in the sense employed by Albright (7) to describe a clinical condition characterized by periods of amenorrhea interrupted by endometrial oozing or hemorrhage occurring without periodicity, and an absence of ovulation.

After these studies were made, definitive therapy for the thyroid disease was started. In those cases in which pregnanediol excretion and endometrial biopsies indicated that normal ovulation had occurred before treatment, no similar post-treatment studies were made. When there was evidence of abnormal menstrual function, however, the tests were repeated at a time when the original clinical menstrual abnormality disappeared, even though some of the patients were not euthyroid at that time.

A total of 18 patients with thyrotoxicosis and 10 patients with myxe-

TABLE 1. FINDINGS IN HYPERTHYROIDISM BEFORE AND AFTER TREATMENT

Patient and age	Pre-treatment				Post-treatment endometrial biopsy
	Menses, Cycle	Flow	Pregnanediol, mg./24 hrs.	Endometrial biopsy	
L. McD. 28	N*	S	—	1. Menstrual, post-ovulatory 2. Proliferative +++	Late secretory (1 d. pre-flow)
C. C. 30	N	S	—	Late secretory (2 d. pre-flow)	Menstrual, post-ovulatory
S. A. 33	N	S	—	Mid-secretory (9 d. pre-flow)	Unsatisfactory
C. Ch. 29	N	N	—	Menstrual, post-ovulatory	—
M. O. 28	N	S	2.3	Late secretory (2 d. pre-flow)	—
Y. C. 34	N	S	—	Early secretory (10 d. pre-flow)	Late secretory (3 d. pre-flow)
M. McN. 45		O	Absent	Proliferative ++	Deciduous tissue† (1st day of flow)
M. G. 45	N	S	Absent	Proliferative +	Menstrual, post-ovulatory
L. D. 31	N	S	Absent	Mid-secretory (7 d. pre-flow)	—
R. D. 26	N	S	1.9	Mid-secretory (3 d. pre-flow)	—
H. A. 36		O	Absent	Proliferative +	Late secretory (3 d. pre-flow)
M. S. 34	N	S	0.4	Menstrual, post-ovulatory	—
R. C. 45		O	—	Proliferative ++	—
V. A. 35	N	S	1.0	Mid-secretory (1st d. of flow)	Early to mid-secretory (1st day of flow)
D. P. 36	N	S	0.6	—	—
A. S. 23	N	S	—	No endometrium obtainable	—
D. C. 24	N	S	1. 1.2 2. {None a month later	—	—
N. O. 27	N	S	—	Early secretory (5 d. pre-flow)	—

* N = Normal cycle or normal flow.
S = Scanty flow.
O = Amenorrhea.
† = Deciduous tissue is taken as presumptive evidence of a previously normal secretory endometrium.

dema were studied. Post-therapy data were available in 16 of the thyrotoxic patients and in 9 of the myxedematous patients. Of the 18 patients with hyperthyroidism only 1 (H. A.) had a multinodular goiter; the remainder were of the classic Graves' variety.

RESULTS

Table 1 is a chart of the pertinent data collected before and after therapy on the patients with thyrotoxicosis. Only 1 patient had a normal menstrual flow, but she had been receiving antithyroid treatment when first studied. The remaining 17 had menstrual disturbances: 14 had a moderate to marked decrease in the amount of flow over a period of at least two months but usually more than five months; and 3 had amenorrhea of from two to twelve months' duration. Of the 15 patients with cyclic bleeding, 13 apparently flowed from a secretory endometrium, although 2 of these (V. A. and N. O.) showed an inadequate corpus luteum effect, and L. McD. had an anovulatory cycle in one of two periods. Another patient (M. G.) flowed from a proliferative endometrium. Satisfactory biopsy specimens were not obtained from 3 patients, but pregnanediol determinations in 2 of these were indicative of an active corpus luteum in each instance.

Of the 4 patients who failed to ovulate, as indicated by endometrial biopsy and absence of preganediol in the urine, 2 had high values for FSH in the urine. The endometrial biopsies showed an inactive endometrium. A third patient, R. C., was in retrospect, menopausal. The fourth had amenorrhea and an inactive endometrium.

Of the 16 thyrotoxic patients with follow-up data, 14 resumed normal periods within three months after starting on definitive therapy for thyrotoxicosis and all 14 became euthyroid during such therapy. Only R. C., the one patient treated with I^{131}, failed to resume normal menstruation. Three patients (C. C., A. S. and D. C.) had a return of normal periods while still hyperthyroid; the others resumed normal periods when they became euthyroid. There was a decided fall in the urinary gonadotropin excretion in the 2 patients (M. G. and M. McN.) whose initial values had been high. Of the 4 patients who apparently were not ovulating prior to antithyroid treatment, 3 (M. McN., M. G., and H. A.) showed evidence of corpus luteum effect after such treatment.

In Table 2 are results from 10 patients with clinical and laboratory evidence of primary myxedema. These comprised all myxedematous female subjects in or near the premenopausal age group who came under observation, and an additional one studied elsewhere. One patient had amenorrhea, 5 had clinical metropathia hemorrhagica, 2 had menorrhagia, and 2 had normal periods. Four patients (E. G., R. Hr., B. C. and E. T.) were examples of spontaneous myxedema. Three of these had metropathia

TABLE 2. FINDINGS IN MYXEDEMA BEFORE AND AFTER TREATMENT

Patient and age	Pre-treatment			Post-treatment endometrial biopsy
	Menses, Cycle	Flow	Pregnanediol, mg./24 hrs.	Endometrial biopsy	
E. G. 49	MH*		Absent biopsy	Proliferative +	Early secretory (9 d. pre-flow)
B. C. 47	O		Absent	Proliferative +	Early secretory (7 d. pre-flow)
E. T. 36	MH		—	Proliferative ++++	Late secretory (4 d. pre-flow)
F. L. 33	N	P	0.3	1. Proliferative + (15 d. pre-flow) 2. Early secretory (6 d. pre-flow)	Late secretory (1st d. of flow)
J. A. 17	N	N	3.0	No biopsy	—
R. D. 34	N	N	—	Proliferative +++	—
R. H. 31	N	P	0.8	Late secretory (2 d. pre-flow)	—
A. T. 33	MH		Absent	Proliferative ++	Early secretory (8 d. pre-flow)
R. Hr. 43	MH		Absent	Proliferative +	Unsatisfactory
E. S. 27	MH		—	Proliferative ++	Deciduous tissue†

* N = Normal cycle or normal flow.
S = Scanty flow.
O = Amenorrhea.
P = Profuse flow.
MH = Metropathia hemorrhagica.
† = Deciduous tissue is taken as presumptive evidence of a previously normal secretory endometrium.

hemorrhagica and 1 had amenorrhea. None ovulated. In the remaining patients myxedema developed following surgical thyroidectomy for Graves' disease (F. L., J. A., R. D., and R. H.), for Hashimoto's struma (E. S.), and for thyroid cancer (A. T.).

It appeared that of the 10 patients with myxedema, 8 had menstrual abnormalities, 7 showed no evidence of ovulation and 1 demonstrated inadequate corpus luteum effect. Of the 2 myxedematous patients who ovulated normally, one (R. H.) had the disease for only a few weeks and the other (J. A.) was only 17 years of age.

Follow-up data were obtained on 9 of the 10 patients with myxedema. Patients E. G., B. C. and E. S. became euthyroid, resumed normal menses and ovulated after one, two and three months respectively of therapy with desiccated thyroid. Patient E. T., with complicating diabetes and myotonia atrophica, did not completely revert to the euthyroid state clinically, although her BMR and PBI responded. Her menses continued to be irregular, with considerable inter-period spotting, despite the fact that her ovulatory mechanism appeared to be normal.

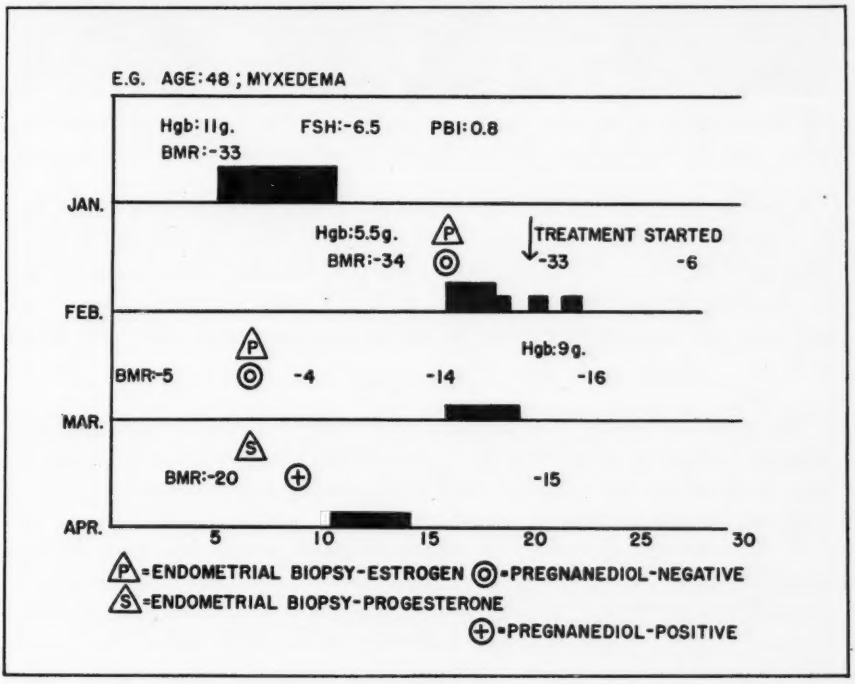

FIG. 1. Menstrual pattern in a patient with myxedema before and after treatment. The symbols are self-explanatory.

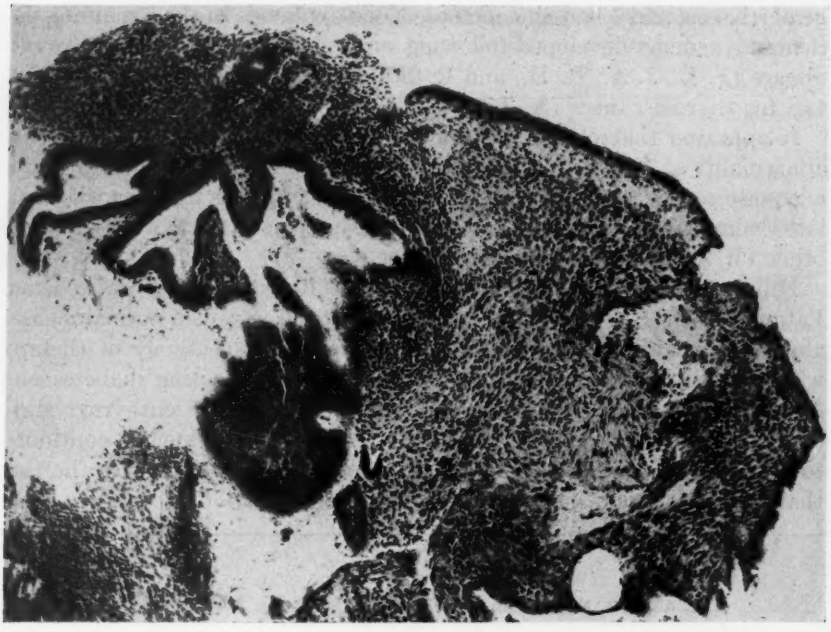

FIG. 2a. Proliferative endometrium from Patient E. G. The biopsy specimen was obtained while the patient was myxedematous and just prior to a profuse menstrual period.

The typical menstrual pattern in a patient with myxedema is illustrated in Figure 1. Photomicrographs of the endometrium in this patient are illustrated in Figure 2a, representing the proliferative endometrium prior to a profuse period before treatment, and in Figure 2b representing a normal secretory endometrium prior to the first normal period after treatment.

DISCUSSION

The results indicate that patients with thyrotoxicosis frequently have oligomenorrhea without ovarian or endometrial abnormalities which can be demonstrated by the technics employed in this study. However, 4 patients had amenorrhea, decreased estrogenic activity, and ovulatory failure, and 2 of these showed increased excretion of gonadotropic hormone. It is of possible significance that 3 of these patients were in an age group when the natural menopause may occur, and they may have responded in an atypical manner because of subtle changes already occurring in the ovary. In all, the menstrual irregularities improved extremely rapidly, usually within one or two months after the start of antithyroid medication. It would seem that there is a critical thyroxine level below which the menstrual

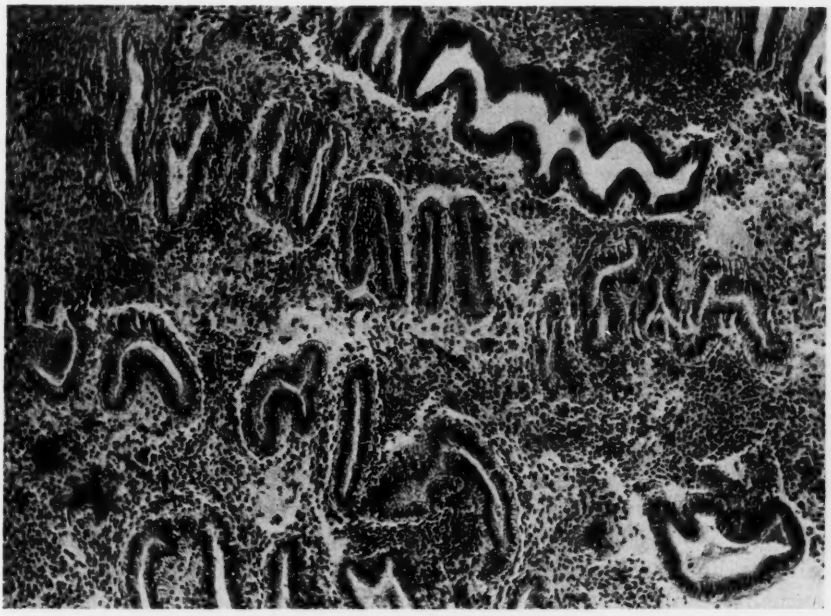

FIG. 2b. Secretory endometrium, after Patient E. G. became euthyroid. The biopsy specimen was obtained shortly before a normal menstrual period.

abnormalities disappear, since some of our patients were still thyrotoxic when the periods became normal.

The nature of the menstrual abnormalities in thyrotoxicosis is not obvious from these studies. It has been suggested that excessive amounts of thyroid hormone depress ovarian function (2). This would explain the low estrogen activity, the failure of ovulation, and the high gonadotropin excretion found in 2 of our patients. In the rat, however, Chu and You (8) found that the feeding of desiccated thyroid hormone decreased the production of FSH and increased that of LH. Our data are consistent either with diminished reactivity of the endometrium, or with partial destruction of the circulating ovarian hormones. The evidence from 2 patients also suggests that this effective decrease in hormone is exaggerated in the older age group, so as to simulate the menopause.

In patients with athyreotic myxedema there often develop irregular, acyclic bleeding or amenorrhea and findings which are indicative of failure of ovulation. This was true in 6 of the 10 patients of this series; a seventh had regular cycles but ovulatory failure. Only 2 had normal physiologic menstruation. One of these patients was only 17 years old. It appears, therefore, that the myxedematous subject is prone to show an abnormal-

ity characterized by a continued estrin effect on the endometrium, but without ovulation and the resulting additional progesterone effect which characterizes the normal cycle.

In spite of the profound disturbance in gonadal function occurring in myxedema, the rapidity of the rebound to normal following adequate substitution therapy was remarkable. It should be noted that in some instances the menstrual abnormalities in myxedema became established only after the disease had been present for months or years, whereas the return to normal on thyroid medication was rapid in almost all cases.

The cyclic regularity and amount of menstrual flow from a secretory endometrium depend upon the functional integrity of the pathways from the anterior pituitary to the ovary and from the ovary to the uterus. Disturbance at any point or combination of points may result in menstrual abnormalities of rhythm, of quantity of flow, and of endometrial structure. The characteristic feature of these myxedematous subjects was ovulatory failure with a continuous estrin effect on the endometrium, leading to the syndrome of metropathia hemorrhagica. Defects in FSH production, ovarian responsiveness to FSH, or effectiveness of estrin on the endometrium would seem to be excluded as factors in the menstrual abnormality. Rather, the defect must have been either in the production of luteinizing hormone or in its effectiveness in the stimulation of ovulation and subsequent release of progesterone. The defect could not have been simply a failure of the endometrium to respond to progesterone, because no pregnanediol was excreted. We have preliminary data suggesting that these patients will respond to injected chorionic gonadotropin, with formation of progesterone and a secretory endometrium. If confirmed, this would place the defect in the myxedematous patient in the LH-producing cells of the anterior pituitary, and suggests that thyroid hormone is a necessary adjuvant in the proper functioning of these cells.

SUMMARY

1. Seventeen of 18 presumably premenopausal female patients with thyrotoxicosis had oligomenorrhea or amenorrhea. Two of these were found to be flowing from a proliferative endometrium. The 3 with amenorrhea had evidence of ovulatory failure and hypoestrinism. One of the patients with amenorrhea subsequently was found to be menopausal. The other 13 showed evidence that, in spite of a scanty or irregular flow, ovulation was occurring and, therefore, there was no major interference with the pituitary-ovarian axis. One patient was lost to follow-up. The remaining 16 patients resumed a normal menstrual pattern after appropriate therapy for the thyrotoxicosis.

2. Seven of 10 unselected premenopausal myxedematous female patients

demonstrated ovulatory failure and an eighth showed an inadequate corpus luteum effect on the endometrium. The remaining 2 patients had normal ovulation and normal menses. All resumed a normal pattern after receiving desiccated thyroid. The abnormalities were considered to be a result of inadequate luteinizing hormone production or effectiveness.

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THE NATURE OF THE SERUM IODINE AFTER LARGE DOSES OF I^{131}*†

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THE administration of a large dose of radioiodine to man (1, 2) and to the rat (3) is sometimes followed by a temporary rise in the level of serum protein-bound iodine. The present study was undertaken to elucidate the nature of the serum iodine after large doses of radioiodine in man.

Recent studies have indicated that the blood iodine normally present in man and in the rat is almost entirely thyroxine, loosely attached to the serum proteins. In support of this view is the demonstration of the identity between the serum iodine and thyroxine added to serum in terms of: precipitability with the serum proteins (4–9), solubility in *n*-butanol (4, 5, 9), inability to dialyze (4, 6, 9), recrystallization to constant specific activity (9), and development in various chromatographic systems (10, 11, 12). The latter method has demonstrated that small amounts of other iodine-containing compounds may also be present. Similar studies have shown that the serum iodine in Graves' disease appears to be identical with thyroxine (13), although there is some evidence which does not support this view (14).

In the present study it was found that the serum iodine in patients with functioning carcinoma of the thyroid, including 3 in whom the normal thyroid tissue had been ablated, was also indistinguishable from thyroxine. In the serum of some patients who were given large doses of radioiodine, however, an iodine-containing substance was found which was not thyrox-

Received for publication November 30, 1951.

* Presented in part at the Annual Meeting of the Association for the Study of Internal Secretions, Atlantic City, N. J., June 7–9, 1951.

† This work was supported by an institutional grant from The American Cancer Society, an institutional grant from the Damon Runyon Memorial Fund for Cancer Research, and in part by a grant-in-aid from the American Cancer Society. It also received support by a grant from the National Cancer Institute of the U. S. Public Health Service and a contract from the A.E.C. No. AT (30-1)-910. The I^{131} was obtained from Oak Ridge.

** Damon Runyon Clinical Research Fellow recommended by the Committee on Growth of the National Research Council, administered by the American Cancer Society.

TABLE 1. SERUM IODINE LEVELS AFTER "TRACER" DOSES OF I^{131}

Case No.	Diagnosis	I^{131} dose (mc)*	Days after dose	Total serum I^{131} (% of dose per liter)	$Zn(OH)_2$-precipitable serum I^{131} (% of total serum I^{131})	Butanol-soluble serum I^{131} (% of total serum I^{131})
A. *Hyperthyroid* (without carcinoma of the thyroid)						
1	Graves' disease	0.456	1	0.168	79.1	89.4
			4	0.403	103	87.2
4	Acromegaly; Graves' disease	0.770	13	1.45	105	84.2
17	Graves' disease	0.528	1	0.345	94.6	81.1
			4	0.584	95.3	82.0
B. *Euthyroid* (without carcinoma of the thyroid)						
7	Malignant melanoma; normal thyroid gland	1.50	2	0.131	144	76.4
			4	0.0774	97.6	83.7
			5	0.0839	—	79.0
18	Metastatic carcinoma of breast; normal thyroid gland	0.685	5	0.109	92.9	86.3
C. *Carcinoma of the thyroid*†						
9	Athyreotic; functioning bone metastases	0.820	10	2.25	—	79.5
10	Athyreotic; functioning lung & neck metastases	2.02	1	1.47	91.1	48.4
			2	1.84	94.6	52.5
			4	2.56	97.0	57.4
12	Athyreotic; functioning lung metastases	2.02	1	1.46	108	70.5
			2	1.59	99.0	71.7
			4	1.84	97.5	72.8
15	Euthyroid; nonfunctioning lung metastases; intact thyroid gland	1.00	3	0.340	—	82.4
19	Athyreotic; nonfunctioning lung metastases	0.555	2	0.719	—	74.2
20	Euthyroid; functioning lung, liver & neck metastases Previous hemithyroidectomy	0.740	6	1.07	94.0	84.5

* Millicuries.

† Of the patients listed as "athyreotic," Nos. 9 and 19 had had total surgical thyroidectomy, No. 10 had had partial surgical thyroidectomy, and Nos. 9, 10 and 12 had had large doses of I^{131} at least one year prior to the present study.

ine. The presence of this material in the blood could be correlated with the destructiveness of the radioiodine on the thyroid tissue.

METHODS

Twenty patients, receiving a total of 27 doses of radioiodine, were studied. Five patients had Graves' disease. Three patients had no thyroid disease. One patient had a nontoxic nodular goiter. Eleven patients had carcinoma of the thyroid. The functional states of the thyroid glands and of the thyroid carcinoma metastases are recorded in Tables 1 and 2. In all of the patients with carcinoma of the thyroid, pathologic examination of biopsy material, either from the primary tumor or from a metastasis, revealed the presence of follicular components in the carcinoma.

The radioiodine (I^{131}) was administered orally in the form of iodide, containing 10 micrograms or less of carrier iodide (I^{127}). Doses of 2.0 millicuries or less were classified as "tracer" doses. "Therapeutic" doses ranged from 8.9 to 254 millicuries. These "therapeutic" doses were being used to treat patients with hyperthyroidism, to destroy functioning metastases from carcinoma of the thyroid, to destroy the normal thyroid gland in patients with nonfunctioning metastases from carcinoma of the thyroid, to produce hypothyroidism in a patient with angina pectoris and in a patient with malignant melanoma, and to reduce the size of a large nontoxic nodular goiter.

Seven patients were studied after "tracer" and after "therapeutic" doses.

Serum obtained at varying intervals after the administration of radioiodine was measured for total radioactivity with a gamma-ray Geiger-Mueller counter equipped with a sensitive Texas Company counting tube (15). The counting rate of the various samples was compared under identical geometric conditions with that of an aliquot of the radioiodine solution from which the dose had been prepared. Calculation for the sample was made in terms of per cent of the administered dose of radioiodine per liter of serum.

Precipitable radioiodine (protein-bound I^{131}) in the serum was determined by measuring the radioactivity of the washed precipitate from 3–6 cc. of serum after treatment with the Somogyi reagents (16). Calculation was made in terms of per cent of the total serum radioiodine.

Butanol-soluble radioiodine in the serum was determined by extracting 2.5 to 5.0 cc. of serum with *n*-butanol, twice with a triple volume, and once with a double volume. The butanol extract received no further treatment. The butanol-soluble radioiodine and the butanol-insoluble residue were measured separately for radioactivity and the percentage of the total serum radioiodine which was butanol-soluble was calculated.

TABLE 2. SERUM IODINE LEVELS AFTER "THERAPEUTIC" DOSES OF I^{131}

Case No.	Diagnosis	I^{131} dose (mc)*	Days after dose	Total serum I^{131} (% of dose per liter)	$Zn(OH)_2$-precipitable serum I^{131} (% of total serum I^{131})	Butanol-soluble serum I^{131} (% of total serum I^{131})	Serum stable iodine (PBI) (γ%)	Approximate radiation to thyroid tissue during second day (repβ)
A. *Hyperthyroid* (without carcinoma of the thyroid)								
1	Graves' disease	9.50	0	—	—	—	5.5	1,680
			2	0.309	92.8	77.0	—	
			4	0.472	86.1	81.7	8.8	
			6	0.613	85.9	83.8	5.8	
			9	0.668	—	81.6	8.7	
2	Graves' disease	8.9	1	0.351	—	76.4	—	1,880
3	Graves' disease	16.9	2	1.38	—	78.3	—	2,180
4	Acromegaly; Graves' disease	25.2	3	—	—	—	4.5**	2,870
			6	2.09	98.8	84.1	6.1	
			17	0.962	100	77.9	5.6	
			24	0.735	97.3	73.3	—	
			31	0.495	95.4	71.1	—	
B. *Euthyroid* (without carcinoma of the thyroid)								
5	Nodular goiter	74.8	0	—	—	—	6.7	2,390
			1	0.124	29.6	80.7	10.7	
			3	0.0649	82.6	72.9	—	
			5	0.0803	82.5	74.3	8.4	
			7	0.0866	80.0	73.2	6.0	
			10	0.0818	59.4	72.0	—	
			15	0.0851	84.5	61.7	11.8	
			22	0.0772	83.9	55.6	—	
			29	—	—	—	7.8	
6	Angina pectoris; normal thyroid gland	103	3	0.0851	45.1	73.7	—	4,910†
			5	0.0519	77.7	66.7	—	
			7	0.0560	78.5	63.0	14.6†	
			10	0.0674	86.3	60.8	—	
			13	0.0648	84.6	51.5	11.7	
7	Malignant melanoma; normal thyroid gland	99.6	0	—	—	—	5.2	33,800
			2	0.117	28.2	81.6	—	
			4	0.0620	54.1	79.9	—	
			6	0.0636	74.9	72.1	5.6	
			9	0.219	75.5	65.8	18.0	
			11	0.188	82.1	67.0	15.3	
			14	0.103	87.7	67.0	—	
			17	0.0634	88.7	63.7	—	
			20	0.0463	86.0	59.9	—	
C. *Carcinoma of the thyroid*‡								
8	Athyreotic; functioning bone metastases	78.5	0	—	—	—	—	2,740
			5	3.64	104	85.2	—	
			7	3.56	98.5	85.4	2.9	
9	Athyreotic; functioning bone metastases	182	0	—	—	—	4.9	3,620
			4	2.29	92.2	69.5	—	
			6	2.54	94.2	65.0	3.8	
			8	2.52	94.9	67.9	4.1	
			16	1.27	—	67.6	—	

TABLE 2—(*continued*)

Case No.	Diagnosis	I^{131} dose (mc)*	Days after dose	Total serum I^{131} (% of dose per liter)	$Zn(OH_2)$-precipitable serum I^{131} (% of total serum I^{131})	Butanol-soluble serum I^{131} (% of total serum I^{131})	Serum stable iodine (PBI) (γ%)	Approximate radiation to thyroid tissue during second day (repβ)
10	Athyreotic; functioning lung & neck metastases	107	0	—	—	—	2.4	5,120
			2	2.45	88.8	43.3	—	
			4	2.94	90.7	41.5	2.6	
			6	2.84	90.8	45.0	1.9	
			9	2.80	92.2	49.0	4.5	
			15	2.33	95.1	48.5	—	
			24	1.38	94.7	41.1	—	
			28	1.20	96.5	38.1	2.5	
11	Athyreotic; functioning lung metastases; nonfunctioning bone metastases	150	4	1.10	86.7	58.5	0.6	6,410
			7	1.31	92.3	49.2	1.0	
			9	1.01	86.7	54.0	—	
			11	0.863	87.8	56.0	0.4	
12	Athyreotic; functioning lung metastases	107	0	—	—	—	1.5	9,630
			2	0.546	91.7	59.0	—	
			4	0.858	83.4	50.0	5.1	
			6	1.00	82.4	47.9	2.1	
			9	1.26	86.7	48.4	2.8	
			15	1.13	88.9	47.5	4.3	
			24	0.599	94.0	47.8	—	
			28	0.496	94.3	43.6	—	
13	Euthyroid; functioning skull metastases; intact thyroid gland	247	7	1.60	89.6	14.1	23.7	16,200
			13	0.724	94.0	21.7	13.2	
			18	0.361	87.3	25.4	—	
14	Euthyroid; minimally functioning neck & lung metastases; previous hemithyroidectomy	50.5	0	—	—	—	4.5	17,700
			1	0.376	72.6	67.0	—	
			3	1.38	92.2	32.1	7.6	
			6	2.55	84.5	40.0	—	
			9	1.55	86.3	54.9	1.8	
			14	0.816	89.5	66.5	—	
			22	0.403	98.8	66.7	—	
			25	—	—	—	2.2	
15	Euthyroid; nonfunctioning lung metastases; intact thyroid gland	70.0	0	—	—	—	4.6	18,000
			4	0.214	—	60.6	—	
			6	0.232	—	45.6	—	
			8	0.298	—	45.0	11.9	
			10	0.188	—	54.4	—	
			12	0.150	—	56.5	10.6	
16	Hyperthyroid; hyperfunctioning lung metastases; functioning neck metastases; previous hemithyroidectomy	254	0	—	—	—	12.6	19,200
			4	—	—	—	60.0	
			12	1.93	97.4	12.3	37.6	
			34	0.131	108	32.2	8.3	
			42	0.0850	105	27.0	5.0	
			62	—	—	—	2.2	

* Millicuries.

** Hyperthyroidism had been controlled by an antithyroid drug prior to the radioiodine therapy.

† In Case 6 the patient had received Priodax for gall bladder visualization one month prior to the radioiodine therapy. The thyroid gland contained only 5 per cent of the administered I^{131} at forty-eight hours after the dose. A control serum stable iodine level was not obtained.

‡ Of the patients listed as "athyreotic," 3 (Cases 8, 9 and 11) had received large doses of I^{131} subsequent to total thyroidectomy. Two (Cases 10 and 12) were thyroidectomized by I^{131} alone or in combination with partial thyroidectomy. In all instances, the large doses of I^{131} were given at least one year prior to the present study.

Recovery experiments after adding I^{131}-labeled thyroxine, diiodotyrosine, and iodide to normal serum were carried out with this method.[1] The butanol extract was found to contain 85 per cent of added thyroxine, 40 per cent of added diiodotyrosine, and 80 per cent of added iodide. The latter two substances were incubated with the serum at approximately 30° C. for twenty-four or seventy-two hours with no significant alteration in their recovery.

I^{131}-labeled thyroglobulin was prepared by macerating the thyroid gland of a rat injected twenty-four hours previously with radioiodide, and extracting with cold 0.9 per cent saline. This extract was assumed to contain chiefly thyroglobulin, and small amounts (1 per cent or less) of thyroxine, diiodotyrosine, and iodide (17, 18). When added to normal human serum, 1 per cent of this material was found to be butanol-soluble. This recovery was not altered by incubation at 30° C. for as long as sixty-four hours.

In 8 patients, the butanol-soluble fraction of serum obtained after "therapeutic" doses of radioiodine was analyzed by paper chromatography. An aliquot of the butanol extract was evaporated to approximately one tenth of its volume by heating in a water bath at about 70° C. under a stream of nitrogen. This material was then transferred to narrow strips of Fisher, E & A qualitative paper (approximately 2 cm.×50 cm.) by streaking several applications in a narrow band near one end. The chromatograms were developed at room temperature in one direction by the ascending technique in each of two solvent systems, one consisting of 70 per cent *n*-butanol and 30 per cent primary amyl alcohol, equilibrated with 2N ammonium hydroxide (19), the other consisting of 80 per cent phenol in aqueous solution (20). After developing for about eighteen hours, the paper strips were removed and dried, cut into 1-cm. sections, placed in cup planchettes, and counted in a beta-ray counter equipped with a thin mica-window Geiger-Mueller tube.

The positions of the radioactive bands were compared with the positions of known radioiodide, thyroxine and diiodotyrosine, the latter two substances being identified by the ninhydrin color reaction. The Rf values in the butanol-amyl alcohol-ammonia system were approximately 0.4 for thyroxine, 0.2 for iodide, and 0.0 for diiodotyrosine. The Rf values in the phenol system were approximately 0.9 for thryoxine, 0.7 for diiodotyrosine and 0.1 for iodide.

The butanol-insoluble fraction of serum obtained from 7 patients after they had received "therapeutic" doses of radioiodine was hydrolyzed with

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[1] I^{131}-labeled thyroxine and diiodotyrosine were obtained from Abbott Laboratories, North Chicago, Ill.

barium hydroxide and extracted with butanol in a modification of the methods of Blau (21) and Perlman *et al.* (22). In 1 patient, the sodium hydroxide method of Leland and Foster (23) was used. Aliquots of the butanol and the alkaline-aqueous fractions so obtained were measured for radioactivity, and calculated in terms of per cent of butanol-insoluble serum radioiodine.

In 5 instances, after barium hydroxide hydrolysis, the hydrolysate was also studied by paper chromatography. A small aliquot of the hydrolysate was acidified with sulfuric acid and the supernatant clear fluid was chromatographed as described above.

Total serum stable iodine and protein-bound serum stable iodine were determined by a modification of the method of Barker (24, 25).

The percentage of the administered dose of I^{131} excreted in the urine, and that retained in the thyroid gland, was measured with the methods described by Hill *et al.* (15).

In those patients who received "therapeutic" doses of radioiodine, the approximate radiation delivered to the thyroid tissue during the second twenty-four hours after the administration of I^{131} was calculated with the formulae of Marinelli *et al.* (26). The fraction of the administered radioiodine which was retained in the thyroid tissue at this time was considered to be equivalent to the fraction not excreted in the urine during the first forty-eight hours after the dose. In some of the patients with intact thyroid glands, the direct measurement of thyroidal radioiodine by *in vivo* counting was considerably smaller than that determined indirectly from the urine data. Since direct *in vivo* measurement of retained radioiodine was not possible in the patients with functioning metastases, however, the indirect method was used in all. The estimate of thyroid tissue weight was based on palpation and x-ray findings. The radiation delivered by gamma rays was disregarded, but would be somewhat larger for thyroid tumors of large mass, than for the thyroid gland. Despite the obvious limitations in these calculations, they were felt to be of value in establishing an order of magnitude for the radiation dosages, and for the rate of their delivery, at a time preceding the extensive destruction of thyroid tissue which sometimes occurred.

RESULTS

The findings in the serum of patients who received "tracer" doses of radioiodine are recorded in Table 1. The precipitable radioiodine accounted for more than 90 per cent of the total serum radioiodine, when measured later than one day after the I^{131} was administered. The butanol-soluble fraction contained from 70 to 89 per cent of the total in all but 1 patient (Case 10), who had a maximum of 57 per cent on the fourth day after the dose was administered.

The data following "therapeutic" doses of radioiodine are recorded in Table 2. The 4 hyperthyroid patients, 1 of the euthyroid patients (Case 5) and 1 patient with carcinoma of the thyroid (Case 8) were found to have butanol-soluble radioiodine fractions similar to those seen after "tracer" doses. The remaining patients who received "therapeutic" doses of radioiodine were found to have smaller butanol-soluble fractions than those observed following the "tracer" doses. If the day on which the total serum radioiodine attained a maximum value (the fourth to tenth day after the I^{131} was administered) is taken as a reference point for these patients, it may be seen that the butanol-soluble radioiodine varied from 14 to 66 per cent of the total serum radioiodine. In 1 patient (Case 16) the serum was not studied at the time of the peak, but on the twelfth day after the radioiodine was administered only 12 per cent of the serum radioiodine was butanol-soluble.

In all patients who received "therapeutic" doses of I^{131}, the serum radioiodine was largely precipitable with zinc hydroxide. It may be seen in Table 2, however, that the percentage of the total serum radioiodine which was precipitable was in some patients somewhat less than that found after "tracer" doses of radioiodine.

Chromatographic analysis was carried out on the butanol-soluble radioiodine fractions of serum from 8 patients. The state of the thyroid tissue in these patients included the following: normal thyroid gland (Case 7), Graves' disease (Case 4), normal thyroid gland and minimally functioning carcinoma of the thyroid (Case 14), normal thyroid gland and highly functional carcinoma of the thyroid (Case 13), athyreosis and functioning carcinoma of the thyroid (Cases 8, 9 and 11), and hyperfunctioning carcinoma of the thyroid (Case 16). The patients classified as athyreotic had received large doses of radioiodine subsequent to total surgical thyroidectomy and sufficiently prior to the present study so that it is reasonable to assume that they had no remaining normal thyroid tissue. The serum used for this study was obtained on the sixth to the ninth day after the administration of "therapeutic" doses of I^{131} (approximately the time of the maximum concentration of total serum radioiodine), except in one instance (Case 16), when the serum obtained on the twelfth day was used.

The findings were similar in all patients, regardless of the diagnosis, and a representative pattern is shown in Figure 1. It is seen that the major portion of the butanol-soluble radioiodine was identical with thyroxine. A small amount of radioiodine was found in the iodide band, and may have been the result of breakdown of thyroxine during the procedure (11, 27). In 2 patients, however, the iodide band seemed larger than would be expected from such breakdown. In one (Case 7) the iodide band contained approximately 25 per cent of the butanol-soluble radioiodine and in the

other (Case 13), approximately 40 per cent. In the latter instance, a small aliquot of untreated serum was chromatographed in the butanol-amyl alcohol-ammonia system, and the positions of the radioactive bands were determined by exposing an x-ray film to the paper strip. Although the bulk of the radioactivity remained at the origin, there was a definite radioactive band at the iodide position, and a fainter band at the thyroxine position.

The butanol-insoluble radioiodine fraction of serum from 8 patients who

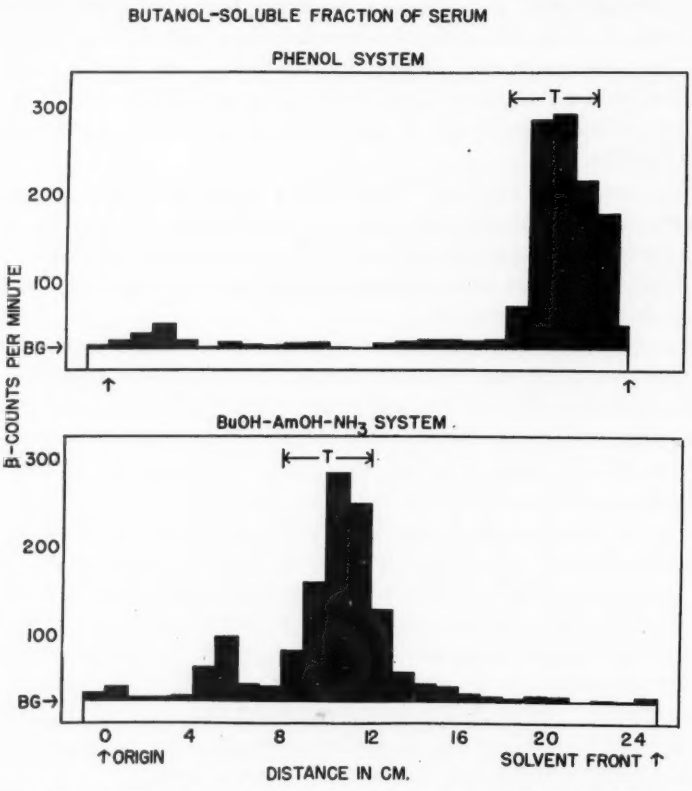

FIG. 1. *Paper chromatography of butanol-soluble serum radioiodine (Case 14).* The graphs represent the radioactivity in 1-centimeter segments of the paper strips. The blackened area represents the radioactivity in excess of background (BG). The upper graph was obtained with 80 per cent phenol in water as the developing solvent; the lower graph, with 70 per cent *n*-butanol and 30 per cent primary amyl alcohol, equilibrated with 2N ammonium hydroxide. The areas designated by "T" represent the positions of known thyroxine on paper strips which were developed simultaneously with the unknowns. The unlabeled peaks at Rf 0.1 in the upper graph and Rf 0.2 in the lower graph are in positions ordinarily occupied by iodide.

had received "therapeutic" doses of I^{131} was subjected to alkaline hydrolysis and fractionation. Two of these patients (Cases 14 and 15) had normal thyroid tissue in the presence of nonfunctioning or minimally functioning carcinoma of the thyroid; the others (Cases 9, 10, 11, 12, 13, and 16) had functioning carcinoma of the thyroid. The serum was obtained at the same times as indicated above for the butanol-soluble fraction. The hydrolysate in these instances contained a "thyroxine-like" substance (soluble in butanol but not re-extracted with the alkaline reagent) which varied

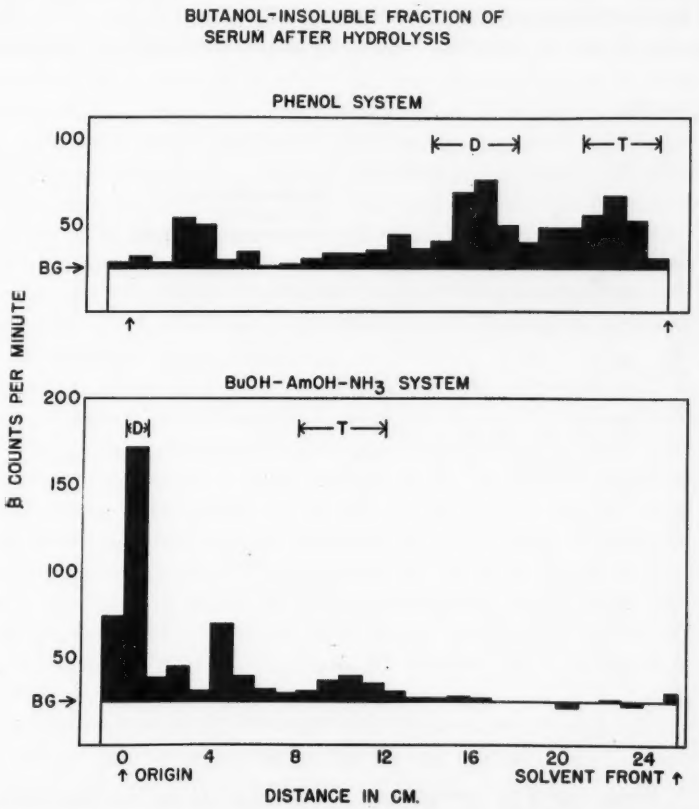

FIG. 2. *Paper chromatography of butanol-insoluble serum radioiodine after hydrolysis with barium hydroxide (Case 14).* The graphs were prepared in the same way as those in Figure 1. The areas designated by "T" and "D" represent the positions of known thyroxine and diiodotyrosine, respectively, on paper strips developed simultaneously with the unknowns. The unlabeled peaks at Rf 0.15 in the upper graph and Rf 0.2 in the lower graph are in positions ordinarily occupied by iodide. The significance of the other bands of radioactivity which may be seen in these graphs is not certain.

between 9 and 33 per cent of the butanol-insoluble material, and averaged 22 per cent.

Chromatographic analysis of the alkaline hydrolysate (prior to extraction) was performed in 5 instances (Cases 9, 10, 11, 13 and 14) with comparable findings in all. A representative pattern is shown in Figure 2. Three bands of radioiodine were found, corresponding in their positions to thyroxine, diiodotyrosine, and iodide, with a large proportion of the radioactivity in the diiodotyrosine position. A fourth band was found just below the diiodotyrosine position in the phenol system (Rf 0.5) and might have represented monoiodotyrosine (28).

The size of the "therapeutic" doses of radioiodine and the approximate

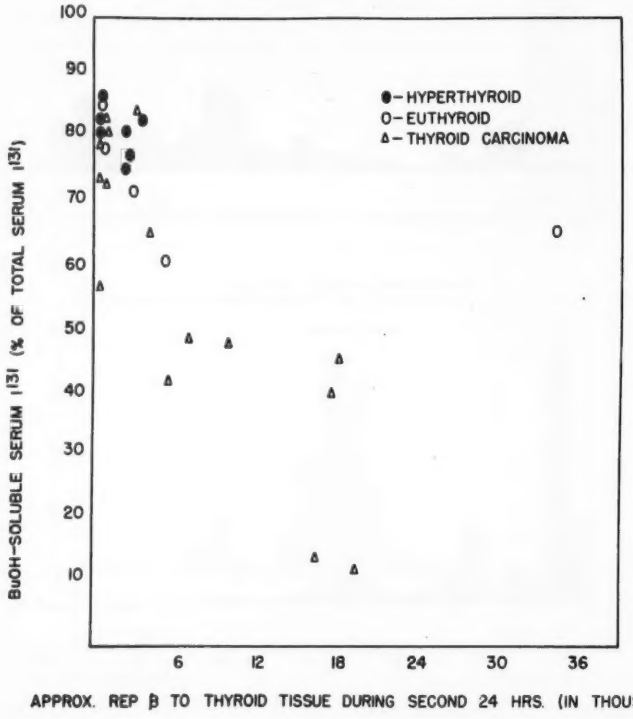

FIG. 3. *Relationship between butanol solubility of serum radioiodine and radiation delivered to thyroid tissue.* The points represent the butanol-soluble fraction of the serum radioiodine at about the time of the peak value in the total serum I^{131}, plotted against the approximate beta radiation delivered to the thyroid tissue during the second twenty-four hours after administration of I^{131}. The points grouped along the ordinate represent the values in patients who were given "tracer" doses of I^{131}; in all of these patients, the radiation delivered during the second twenty-four hours was less than 300 repβ.

beta radiation delivered to the thyroid tissue during the second twenty-four hours after these doses, are recorded in Table 2. It may be seen that a depression in the butanol-soluble serum radioiodine fraction occurred in those patients who received an estimated 3600 repβ or more during the second twenty-four hours. The correlation between these two factors is shown graphically in Figure 3. The lack of correlation between the amount of I^{131} administered and the amount of radiation delivered is worthy of emphasis. This is due to differences in accumulation of radioiodine and, in larger part, to differences in the size of thyroid tissue. For example, one patient (Case 5) in whom a dose of 75 millicuries of I^{131} delivered only 2400 repβ, had a very large nodular goiter, estimated to weigh 200 grams.

The values for the protein-bound stable iodine in the serum after large doses of radioiodine are also recorded in Table 2. In some patients there was a rise in the stable PBI accompanying the rise in serum radioiodine. In one patient (Case 16) this rise was to an extreme degree (60 micrograms per cent). It may be seen that there was no direct correlation between the occurrence of an elevation in the level of stable PBI and the presence of abnormal serum radioiodine fractions or the amount of radiation delivered. The total stable iodine in the serum, which is not recorded in the table, paralleled the PBI, there being no instance of a significant increase in non-precipitable serum stable iodine.

The daily urinary excretion of radioiodine after "therapeutic" doses of I^{131} was measured for two weeks or longer in all but 3 patients (Cases 2, 3 and 4). With the exception of 2 patients (Cases 6 and 9), those who had a depression in the serum butanol-soluble radioiodine fraction also had an abrupt increase in the daily radioiodine excretion, occurring within a few days prior to the peak in the total serum radioiodine. This increase occurred on the fourth or fifth day following the dose, except in 2 patients (Cases 7 and 15), in whom the change occurred on the seventh day. A representative example of this finding is seen in Figure 4. In the other patients so studied (Cases 1, 5, 6, 8 and 9), the urinary excretion followed a smooth curve.

In 4 patients (Cases 6, 7, 14 and 15) frequent *in vivo* measurements of radioactivity over the thyroid gland were made. In 3 (Cases 7, 14 and 15) there was an abrupt fall in thyroidal radioiodine coincident with the peak rise in serum radioiodine. This finding is also illustrated in Figure 4.

DISCUSSION

The serum iodine under normal circumstances and in hyperthyroidism has been shown to be almost completely precipitable by agents which cause precipitation of the serum proteins (4–9), and almost entirely extractable with *n*-butanol (4, 9). Washing the butanol extract with alkali re-

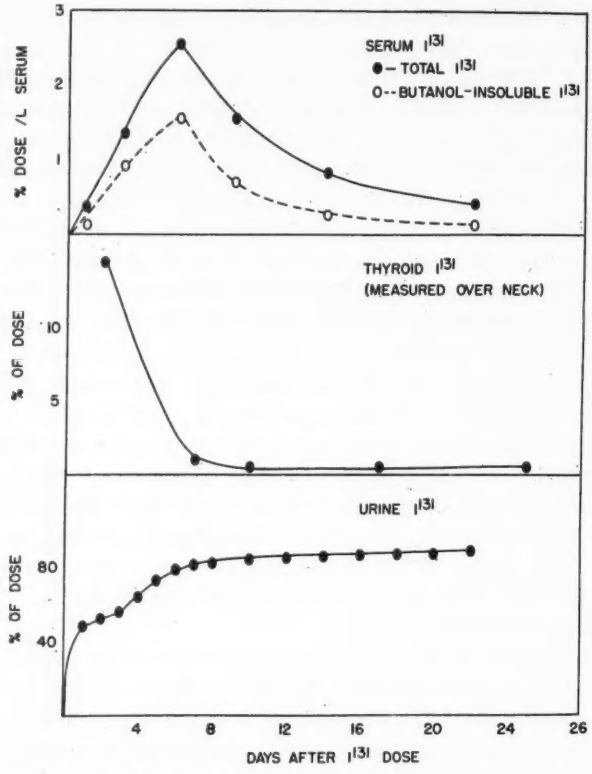

FIG. 4. *Relationship between serum I^{131}, thyroidal I^{131} and urinary I^{131} after a large dose of radioiodine (Case 14).* The urinary I^{131} is plotted as cumulative excretion.

moves some of the butanol-soluble iodide (5, 29, 30). Studies on serum radioiodine after administration of "tracer" and "therapeutic" doses of radioiodide (31) indicated that in normal subjects almost all of the serum I^{131} was precipitable in about ninety-six hours, and in hyperthyroid subjects, in about forty-eight hours. Butanol extraction of serum, however, at a time when all of the radioiodine was precipitable, removed only about 80 per cent of the total serum I^{131} (9, 13, 32). Rosenberg (13), on the other hand, in studying the blood radioiodine in hyperthyroid patients after "therapeutic" doses of I^{131}, found that butanol extraction of a trichloracetic acid precipitate of serum resulted in recovery of practically all of the serum radioiodine in the butanol fraction. Since direct butanol extraction of serum results in a sticky precipitate which is broken up with difficulty, it would seem that the discrepancy may be due to limitations of the direct extraction method. Another possibility is that butanol extraction of plasma

is more complete when carried out at an acid pH, as was done by Rosenberg.

The findings herein reported on the serum radioiodine after "tracer" doses of I^{131} are in essential agreement with the results of other investigators. The observations in the hyperthyroid patients who received "therapeutic" doses of I^{131} confirm the results reported by Rosenberg. The serum radioiodine in these patients resembled that seen after "tracer" doses, in terms of precipitability with zinc hydroxide and solubility in butanol. Chromatography of the butanol-soluble fraction indicated that most of the serum radioiodine was in the form of thyroxine.

In 1 patient with nontoxic nodular goiter and in 1 patient with functioning carcinoma of the thyroid, the serum radioiodine after "therapeutic" doses of I^{131} was again similar to that seen after "tracer" doses. In the remaining patients who received "therapeutic" doses, the butanol-soluble fraction was found to be unusually low, although the precipitable fraction still accounted for most of the serum radioiodine.

When the butanol-soluble radioiodine was studied by paper chromatography, it was found to consist almost entirely of a substance resembling thyroxine, except for 2 instances in which 25 to 40 per cent of the butanol-soluble radioiodine resembled iodide. It was of particular interest that in those patients with functioning carcinoma of the thyroid in whom normal thyroid tissue was presumably absent, the butanol-soluble serum radioiodine was indistinguishable from thyroxine.

The chromatographic analyses also indicated that diiodotyrosine was not present in the butanol-soluble fraction in an amount large enough to account for the increase in butanol-insoluble radioiodine. Diiodotyrosine would be largely precipitable with zinc hydroxide (7, 8) and partially insoluble in butanol.

The findings, therefore, suggested that in the serum of some patients following large doses of I^{131} there was present iodine-containing material which was neither thyroxine, diiodotyrosine, nor iodide in a free or "loosely bound" form. After alkaline hydrolysis, however, the butanol-insoluble fraction released three iodine-containing components which, on chromatographic analysis, resembled thyroxine, diiodotyrosine, and iodide. Furthermore, the relative amounts of these components and the finding that an average of 22 per cent of the hydrolysate resembled thyroxine in terms of butanol solubility, were suggestive of the composition of thyroglobulin as determined after alkaline hydrolysis (21, 23, 33, 34). The presence of a fourth component which might have been monoiodotyrosine is also compatible with the composition of thyroglobulin, since this compound has been demonstrated in thyroid gland hydrolysates (28). Further studies are necessary to determine whether the butanol-insoluble fraction of the

serum actually contained thyroglobulin (or fragments of the thyroglobulin molecule).

The findings in 3 patients of a marked fall in thyroidal radioiodine, accompanying the appearance of butanol-insoluble radioiodine in the serum, supports the belief that the material resulted from destruction of thyroid tissue. Similar observations have been made in rats by Feller *et al.* (3), who studied stable iodine in the thyroid glands and serum after large doses of I^{131}. It is of interest to note that these investigators observed an apparent leakage of colloid out of the damaged follicle in histologic sections of the rat thyroids (35).

The apparent direct relationship between the amount of radiation delivered to the thyroid tissue and the presence of butanol-insoluble radioiodine in the serum is pictured in Figure 3. Clinical observation of the patients also revealed a correlation between the abnormal serum fractions and the degree of thyroid tissue destruction. The hyperthyroid patients and the patient with nontoxic nodular goiter had no significant discomfort in the neck following the I^{131} doses. One patient (Case 15) had mild discomfort in the neck. Two patients (Cases 6 and 7) had moderately severe pain and tenderness of the thyroid gland and 2 patients (Cases 13 and 14) had severe radiation thyroiditis. One patient (Case 16) had a dramatically rapid decrease in size of her lung metastases. The other patients with carcinoma of the thyroid had either a gradual diminution, or no change, in the size of their metastatic lesions.

In rats (3) a similar relationship between the amount of thyroid gland radiation and the finding of an elevation in the serum PBI has been demonstrated. This study also showed that a dose of I^{131} capable of completely destroying the thyroid gland would not necessarily cause a rise in serum PBI. It was suggested that the rate of destruction was the determining factor.

The appearance of butanol-insoluble radioiodine in the blood was not always accompanied by a rise in serum stable iodine, and the magnitude of the changes in these two measurements did not necessarily parallel each other. Inasmuch as the rise in serum stable iodine would be dependent on the size of the iodine store in the thyroid tissue, it is of interest to note that of the patients with carcinoma of the thyroid, all except 3 (Cases 14, 15 and 16) had been treated with thiouracil over a prolonged period of time. In these patients, one would anticipate a depletion of stored iodine. Of the patients who had a striking increase in stable PBI, only 1 (Case 13) had received an antithyroid drug.

Hyperthyroidism following the administration of large doses of radioiodine has been described (1, 2). In the present study, the following obser-

vations may be noted: None of the patients with Graves' disease had an exacerbation of his hyperthyroidism following the "therapeutic" dose of I^{131}. The 3 euthyroid patients had clinical evidence of transient mild hyperthyroidism following the large doses, and in each the BMR showed a transient elevation. In none of the patients with carcinoma of the thyroid did hyperthyroidism develop.

One patient (Case 16) is of particular interest because of the remarkable rise in PBI to 60 micrograms per 100 cc. Prior to the "therapeutic" dose of I^{131}, this patient was hyperthyroid, with a PBI of 12.6 micrograms per cent and a BMR of +16. Following the dose, there was no clinical evidence of increasing hyperthyroidism, and on the eighteenth day after the dose the BMR was +7. Since only 12 per cent of the serum radioiodine in this patient was butanol-soluble, it is likely that most of the increase in stable iodine was due to nonthyroxine iodine. Furthermore, it was apparent that this material was not calorigenic. This does not preclude the possibility that the nonthyroxine iodine could be thyroglobulin, since Lerman (36) demonstrated that there was no correlation between postoperative exacerbation in hyperthyroidism and the presence of thyroglobulin in the thyroid venous blood during surgical thyroidectomy.

Maloof and Chapman (37) have observed a rise in serum PBI following "therapeutic" doses of I^{131} in 2 patients with Graves' disease, in whom there was no post-treatment exacerbation in the hyperthyroidism.

It is possible that a large dose of radioiodine may in some patients be followed by a rise in thyroxine iodine, as well as in nonthyroxine iodine, in the serum. The measurement of butanol-soluble stable iodine in the serum would be of interest in this regard.

SUMMARY

The serum radioiodine was studied in 11 patients following "tracer" doses of I^{131}. More than 90 per cent of the serum radioiodine was precipitable with the Somogyi reagents, and 70 to 89 per cent was extractable with *n*-butanol.

In 10 of 16 patients who were studied after "therapeutic" doses of I^{131}, it was found that although the serum radioiodine was largely precipitable, only from 12 to 66 per cent was butanol-soluble. After alkaline hydrolysis, the butanol-insoluble radioiodine fraction was shown to release thyroxine, diiodotyrosine, and iodide in proportions comparable to those found in thyroglobulin. The occurrence of this abnormal material in the blood appeared to be related to the amount of radiation delivered to the thyroid tissue, and was most pronounced in those patients who had the most severe radiation damage.

In some patients, the presence of butanol-insoluble radioiodine in the serum was accompanied by a rise in serum protein-bound stable iodine. This material did not appear to produce a calorigenic effect.

It has also been demonstrated that the serum radioiodine elaborated by functioning carcinoma of the thyroid appears to be identical with thyroxine.

Acknowledgments

The authors are indebted to Mrs. Jean Dorans, Mrs. Audrey Peck, Miss Jane Heslin, Miss Mary Sultzer and Miss Bernadette Leclerc for their most helpful technical assistance.

Addendum

Since this manuscript was submitted for publication, Tong *et al.* (38) have reported results of a similar nature, demonstrating the presence of large amounts of nonthyroxine radioiodine in the plasma of rats after large doses of I^{131}.

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PHEOCHROMOCYTOMA AND ADRENAL CORTICAL ADENOMA. REPORT OF A CASE WITH BOTH TUMORS AND DISCUSSION OF THEIR RELATION*

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INTRODUCTION

THE independent investigations of Vogt (1, 2) and Long (3, 4) from 1944 to 1947 firmly established in laboratory animals a secretory relationship between the adrenal medulla and cortex. Epinephrine released by splanchnic stimulation or injected intravenously is followed by increased cortical secretion. This action of epinephrine has been demonstrated by Long to take place via the anterior pituitary. The epinephrine stimulates the anterior pituitary to release corticotropic hormone (ACTH) which, in turn, stimulates the adrenal cortex. Clinical confirmation of these laboratory studies has been obtained by Thorn *et al.* (5), who have demonstrated that epinephrine causes a depression in circulating eosinophils comparable to that following administration of ACTH (6).

In view of this physiologic relationship between the medulla and cortex of the adrenal gland, now apparently well established, it is curious that patients with a hypersecreting tumor of the adrenal medulla, *i.e.*, a pheochromocytoma, do not commonly exhibit obvious disturbance of adrenal cortical function.

A survey of the literature reveals few instances of possible adrenocortical derangement in patients with a pheochromocytoma. Sprague (7) studied the urinary 11-oxysteroid and 17-ketosteroid excretion in a patient with a pheochromocytoma and found both to be elevated. Neff (8) has reported the case of an infant who showed hypertrichosis, obesity and other features of Cushing's syndrome but who was found to have only a pheochromocytoma. Graham (9) in his recent, extensive review of the clinical manifestations seen in patients with pheochromocytoma lists 4 instances in which there was possible evidence of adrenal cortical hyperactivity in the form of hirsutism, obesity, and scanty menses.

Received for publication January 12, 1952.

* This study was supported in part by a grant from the American Cancer Society to the Massachusetts General Hospital and in part by a contract between the Office of Naval Research and Harvard University for the study of surgical disease.

The simultaneous occurrence of an adrenal cortical adenoma in association with a pheochromocytoma has not been reported. The following case presents the combination of pheochromocytoma and a functioning, adrenal cortical adenoma without, however, clinical or laboratory evidence of hyperadrenocorticism.

CASE REPORT

G. E., a 55-year-old white male, was admitted to the Massachusetts General Hospital on December 27, 1950, complaining of "spells" which first occurred ten years prior to entry. These "spells" generally were of ten to fifteen minutes' duration and consisted of the sudden onset of frontal headache, nausea, palpitation, profuse perspiration, a fluttery feeling in the epigastrium, labored breathing, and generalized trembling with cooling and blanching of the extremities. The "spells" left the patient in an exhausted, sleepy state. The attacks initially occurred at approximately monthly intervals but recently had attained almost daily frequency and for a year prior to entry they had prevented the patient from working. Attacks were more likely to occur before breakfast than at any other time of day; emotional episodes and paroxysms of coughing were thought by the patient to be precipitating factors.

In addition, the patient complained of a loss of libido. There had been an eight-pound weight gain in the three years prior to entry.

Admission physical examination revealed a well developed, husky appearing, middle-aged male with normal distribution and amount of body hair and subcutaneous tissue. The skin was of normal thickness without striae. Retinal arterioles were narrowed and somewhat tortuous. Blood pressure was 144/82; the pulse was regular with rate of 68, and slight cardiac enlargement was noted. Liver, kidney and spleen were not felt and no other abdominal masses could be outlined. Except for prostatic hypertrophy, the remainder of the physical examination was not remarkable.

Initial laboratory studies showed an hematocrit of 51 per cent cells, and a normal white blood cell count and differential. The urine did not show any sugar or albumin, though an occasional, subsequent Benedict's test gave an olive-green reaction with sediment.

Numerous diagnostic studies were carried out preoperatively. Because of the danger of provoking a vascular catastrophe, the histamine test was omitted; neither was a benzodioxan test performed. A postural test showed a drop of 30 mm. systolic and 20 mm. diastolic pressure, but with a decline in pulse rate upon changing to the erect position. The cold pressor test response was insignificant. Other laboratory findings included: B.M.R. −1 per cent; fasting blood sugar 187 mg./100 cc. with a diabetic glucose tolerance curve; and fasting eosinophil count 163/cu. mm. The urinary 17-ketosteroid excretion was 4.2 mg./twenty-four hours. No paroxysmal attack occurred during the collection period.

X-ray studies, including an intravenous pyelogram, revealed a large, rounded soft-tissue mass, containing flecks of calcification, above the upper pole of the right kidney. There was no recognizable osteoporosis.

While on the medical service before operation, the patient was observed during several spontaneously occurring attacks of the nature just described. In one of these episodes, the blood pressure rose to 260/110 with a fall to 150/100 within ninety seconds; the blood sugar level (fasting state) at this time was 96 mg. per cent. Thirty minutes later the blood sugar level was 186 mg. per cent and the serum potassium, 4.8 mEq. per liter.

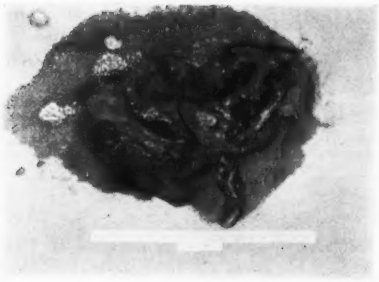

FIG. 1. The double tumor sliced into halves and the cut surfaces exposed. The halves of the larger, greyish-brown, partially cystic pheochromocytoma lie in the center. At either end are the halves of the yellow, solid cortical adenoma. Below the tumor mass near the measuring scale is a portion of the non-neoplastic, atrophic adrenal cortex. This photograph was taken in the operating room immediately after excision. The measuring scale is 15 centimeters long.

The eosinophil count at the onset of this attack was 246/cu. mm., with a fall to 136/cu. mm. four hours later.

On the patient's tenth hospital day, operation was performed. Exposure of the right suprarenal area revealed a rounded tumor mass measuring approximately 12 cm. in diameter. The abundant vascular supply to the mass was ligated with difficulty, an effort being made to avoid expressing hormone into the circulation. In spite of this effort, however, towards the end of the excision of the tumor, the blood pressure rose. The rise was promptly controlled by the intravenous injection of benzodioxan. Immediately following the excision the blood pressure fell to subnormal levels; the intravenous injection of nor-epinephrine was started.

During the dissection of the tumor, the uninvolved portion of the adrenal cortex was visualized and found to be atrophic rather than normal. The edge of the cortex was paper thin and bright yellow. Such thinness and color is seen in the undiseased atrophic portions of the cortex of patients with Cushing's disease due to a hyperfunctioning single adenoma of the cortex. In addition, the top of the tumor was yellowish, more like a cortical than a medullary tumor.

The meaning of the atrophic cortex and yellow dome of the tumor was clear grossly when the tumor was sectioned in the operating room; the tumor consisted of two parts, a larger semi-cystic greyish-brown tumor consistent with a pheochromocytoma, and above it, a smaller yellowish cortical adenoma (Fig. 1).

It was immediately realized that the cortical tumor had been a functioning one and that cortical therapy must be a part of the postoperative management. The reasoning was as follows: the atrophy of the uninvolved portion of the cortex could have been due either to pressure from the tumors or to lack of hormonal stimulation due to suppression of the anterior pituitary by the cortical tumor's secretion. That it was not due to pressure was indicated by comparable atrophy in the tongue of the cortex extending downwards along the vena cava, well removed from the tumor masses. Lack of hormonal stimulation as the only reasonable explanation for the observed cortical atrophy, meant that the cortex of the contralateral adrenal would also be atrophic and that with resection of the cortical tumor there would be a phase of cortical insufficiency following operation. This phase would pass, only as the atrophic cortex regained its functional capacity.

To offset this anticipated phase of hypocorticism, cortisone therapy was started immediately. Moreover, as little as possible of the uninvolved cortex was removed; only that needed to insure complete excision of the tumors was resected. The tongue along the vena cava was left intact; this was estimated to weigh less than 1 gram. A snippet of the excised atrophic cortex was implanted subcutaneously as a graft at the end of the operation.

The patient's early postoperative course was marked by persistent hypotension with the blood pressure falling to 65/42. Cortisone was administered at four-hour intervals, in a dosage of 50 mg., for a total of 200 mg. Nor-epinephrine was given by continuous intravenous drip to maintain a satisfactory level of blood pressure; this required vigilant attention, since pressure response was marked with minimal changes in nor-epinephrine intake.

Thirty-six hours after operation, the nor-epinephrine was discontinued with a subsequent, satisfactory normotensive level being maintained by the patient's homeostatic mechanisms.

Postoperative convalescence was complicated by pulmonary atelectasis and a subdiaphragmatic wound abscess requiring incision and drainage.

The patient was discharged on the twentieth postoperative day. After operation he

was free of paroxysmal attacks; a histamine test performed just prior to discharge showed no rise in blood pressure.

Since discharge, the patient has been followed for ten months in the Surgical Clinic. There have been no attacks and he is otherwise asymptomatic, having returned to his former employment. Blood pressure remains in the vicinity of 130/80.

Examination of the excised specimen by the pathologist (Dr. Benjamin Castleman) confirmed it to be of two portions. The larger mass, weighing 185 grams, was grey, gelatinous and multicystic, measuring 9.5 cm. in diameter, with the largest of the several cysts having a diameter of 5 cm. On cut section, the cysts exuded an oily, golden fluid. Calcification could be seen in the walls of the smaller cysts. Microscopic examination of this large mass confirmed the diagnosis of pheochromocytoma.

The smaller mass of golden hue weighed 25 grams and measured $4.5 \times 4.0 \times 2.5$ cm. Histologically it was a cortical adenoma.

A part of the pheochromocytoma was forwarded to Dr. Marcel Goldenberg of the College of Physicians and Surgeons in New York, who kindly determined the epinephrine and nor-epinephrine content of the tumor (10). It contained 3.8 mg. of epinephrine and 2.8 mg. of nor-epinephrine per gram of tissue.

DISCUSSION

The subject of this report had two tumors of the adrenal gland, both of functioning type.

The pheochromocytoma was hyperfunctioning and the source of the attacks of paroxysmal hypertension. It was found to contain epinephrine and nor-epinephrine. Its removal was followed by relief from the attacks.

The cortical adenoma was functioning to the extent that the uninvolved cortex had atrophied. Its secretion had presumably suppressed the hormonal mechanism normally maintaining the anatomic integrity of the cortex.

Was there an etiologic relation between these two tumors or was their presence purely coincidental? The known stimulating effect of epinephrine upon the adrenal cortex has been reviewed in the introduction. To postulate that the pheochromocytoma had caused the cortical tumor involves several theoretic steps.

Step 1. Madison (11) has shown that it is epinephrine, not nor-epinephrine, which stimulates ACTH release and increases cortical function. Epinephrine was found in abundance in the pheochromocytoma of this patient.

Step 2. The secretion of epinephrine could theoretically have induced hyperplasia of the cortical tissue. ACTH has been found to induce hyperplasia of the cortex in experimental animals.

Step 3. The long-continued, paroxysmal secretion of epinephrine and the intermittent stimulation of the adrenal cortex to hyperplasia might have resulted in adenoma formation among the cells of the cortex.

Step 4. As this adenoma and its secretory capacity grew, its hormone output should eventually have reached a level at which the normal govern-

ing output of ACTH would be suppressed and atrophy of the non-adenomatous cortex ensue.

The observed facts of this case fit such a theory. The question immediately arises, however, as to why cortical hyperplasia or adenomas have not previously been encountered in other patients with a pheochromocytoma. There are several possible explanations for this and they are recounted in the hope of stimulating observation in future cases.

In the first place, perhaps a high concentration of epinephrine is needed in the secretion of the pheochromocytoma. Secondly, the secretory curve of the epinephrine may need to be sufficiently prolonged and intense and the attacks of frequent enough occurrence.

Thirdly, cortical hyperplasia may have been overlooked in some of the reported cases of pheochromocytoma. Cortical hyperplasia is notoriously difficult to define; gross and microscopic criteria are lacking. The adrenal glands without tumor associated with Cushing's disease, now believed to be a form of hyperplasia, have for many years been labeled normal for want of such criteria. In favor of this Cushing's type of cortex being hyperplastic is the observation that when such glands are subtotally resected, the Cushing's disease is promptly relieved. The relief equals that following resection of an adrenal tumor when such is present, and is comparable to that achieved by subtotal resection of the hyperplastic thyroid in Graves' disease and of the hyperplastic parathyroids in hyperparathyroidism.

Fourthly, cortical adenomas may have been overlooked in patients whose pheochromocytomas have been resected surgically. Commonly only one adrenal gland is exposed in resecting the medullary tumor. A small adenoma of the cortex may well have been present in the adrenal of the opposite side. There is no reason why the cortical adenoma should always be in the same adrenal with the pheochromocytoma. Had the cortical adenoma of this patient been in the contralateral adrenal, the only finding which might have brought it to our attention was the cortical atrophy. Such atrophy is also easy to overlook in the haste and attention of resecting the pheochromocytoma.

No claim is made that the cortical adenoma was hyperfunctioning. The normal or supranormal level of the circulating eosinophils (12) and the absence of signs of Cushing's disease are against hyperfunction. The low level of the 17-ketosteroids is consistent however with hyperfunction. A comparable low level has been encountered preoperatively in the majority of 12 patients with Cushing's disease whose adrenal adenomas have been resected surgically at this hospital (13).

SUMMARY

The experimental physiologists have furnished abundant evidence that epinephrine stimulates adrenal cortical activity. Patients with a pheo-

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chromocytoma, however, who show evidence of increased adrenal cortical activity are rare.

A case is presented of a patient with both a pheochromocytoma and a functioning adrenal cortical adenoma. Although the two tumors can only be said to have coexisted, the possibility is discussed that the cortical adenoma was induced by the epinephrine secreted by the pheochromocytoma.

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THE ADMINISTRATION OF PURIFIED GROWTH HORMONE TO A FEMALE WITH HYPOADRENALISM, HYPOTHYROIDISM, DIABETES MELLITUS, AND SECONDARY AMENORRHEA, SIMULATING PANHYPOPITUITARISM

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THE purpose of this paper is, first, to report a case in which the coexistence of diabetes mellitus and hypoadrenalism was further complicated by the presence of hypothyroidism and secondary amenorrhea and second, to report the results of the administration of purified growth hormone to this patient. The incidence of the coexistence of diabetes mellitus and hypoadrenalism in itself is very rare as only 32 cases (1–6) have been reported up to July 1, 1951. It will be brought out in our discussion that this patient did not have panhypopituitarism but rather multiple "end organ failure." A similar case has been reported (7) in a male, but to our knowledge a similar case has not been reported in a female.

CASE HISTORY

W. S., a 19-year-old white female was admitted to the University of Virginia Hospital on October 13, 1949, in a semi-comatose state.

The patient had been known to have diabetes for approximately six years. Her insulin requirement had ranged from 45 to 60 units of protamine-zinc insulin daily. One year previously amenorrhea had developed, with excessive fatigue, malaise, and periods of drowsiness. At this time her insulin was reduced to 30 units of PZI daily. One week before admission she had entered a local hospital with a respiratory infection of two weeks' duration. A diagnosis of virus pneumonia was made, but no antibiotic therapy was given. The patient was found to be hypoglycemic on 30 units of PZI. The dosage was reduced and finally discontinued. Forty-eight hours after the last dose of insulin she was found in a semi-comatose state. The blood pressure was 60/40 and the blood sugar was found to be 33 mg. per cent. A diagnosis of acute hypoadrenalism was made. She was given emergency treatment and referred to the University Hospital for further care.

On admission to the University Hospital she presented signs and symptoms of adrenal failure but responded to the usual intensive emergency treatment.

The following physical findings were of interest: The blood pressure was 60/40, pulse 88, and temperature 97.4°. The face appeared puffy with definite periorbital edema (Fig. 1). The skin was pale, dry and cold with numerous small irregular areas of pigmentation on the arms, legs, and face. There was also pigmentation circumorally, at the

Received for publication November 26, 1951.

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FIG. 1. Patient W. S. Frontal view of face.

mucocutaneous junction of the vagina, and on the buccal mucosa above the right first incisor. The pubic and axillary hair was sparse. The breasts were virginal with no pigmentation of the nipples. Examination of the chest revealed signs of effusion and consolidation at both bases. After she regained consciousness, it was noted that she had a myxedematous type of speech.

The significant laboratory data on admission were: RBC 3.77 million, hemoglobin 13.5 Gm., WBC 9,500 with 39 per cent polymorphonuclear cells, 54 per cent lymphocytes, 6 per cent eosinophils, and 1 per cent basophils, blood sugar 74 mg. per cent, BUN 19 mg. per cent, serum chloride 93 mEq./L, and CO^2 combining power 24 mEq./L. (The patient received an infusion of glucose three hours before these determinations were made.) The urine was acid, with a specific gravity of 1.026 and showed no sugar, acetone, or albumin.

An electrocardiogram showed low voltage, a QT interval of 0.44 and inverted T waves in leads V2, V4, and V5. An electroencephalogram was interpreted as follows: "The cortical activity is mildly abnormal, showing slower frequencies than one would expect to find in a normal tracing." X-ray examination of the skull showed nothing abnormal. The chest examination revealed density at both bases, thought to be a bilateral

pleural effusion, and the cardiac shadow revealed what was thought to be a pericardial effusion.

Numerous laboratory procedures were carried out in order to elucidate the endocrine status. These are discussed under "Comment."

COMMENT

In this patient with known diabetes mellitus there developed a striking sensitivity to insulin, which suggested to the referring physician the presence of hypoadrenalism. The patient also presented features of hypothyroidism and hypo-ovarianism. A number of authors have established the direct relationship between insulin requirement and the dosage of adrenal cortical substitution therapy in the form of adrenal cortical extract, cortisone, and compound F (4, 8, 9). These latter materials were not available to us at the time the patient was studied. However, the relationship between the insulin requirement and the dosage of Lipo-adrenal Extract was evident.

An attempt was made to determine whether the endocrine deficiencies represented multiple "end organ" failure or whether they were secondary to "panhypopituitarism." The following diagnostic features for each system appear to establish that this patient suffered from multiple organ failure:

Adrenal: Hypotension, pigmentation, and hypoglycemia occurring in a known diabetic (without insulin therapy) are all suggestive of hypoadrenalism. Total eosinophil counts of 637, 837, 762, and 569 per cu. mm. were recorded on four separate occasions (10). A fall of 5 per cent in the eosinophil count four hours after the administration of 25 mg. of ACTH and an excretion of 1.7 mg. of 17-ketosteroids per twenty-four hours (normal, 5–15 mg. per twenty-four hours) (11) substantiate the diagnosis of hypoadrenalism.

The following evidence suggests that the hypoadrenalism was "primary": After the administration of 25 mg. of ACTH every six hours for forty-eight hours the total eosinophil count did not fall significantly and the 17-ketosteroid excretion was increased from 1.7 mg. to only 2.5 mg. per twenty-four hours.

Thyroid: The dry, cold, pale skin, characteristic hoarse voice, and serous effusions, plus a serum cholesterol level of 328 mg. per cent, a BMR of −31 per cent, a "low voltage" electrocardiogram, and a radioactive iodine uptake of 12 per cent, establish the diagnosis of hypothyroidism.

The patient was given 25 mg. of TSH[1] (thyroid-stimulating hormone) twice daily for ten days. There was no increase in the radioactive iodine uptake. This was interpreted as evidence of primary hypothyroidism (12).

[1] Supplied through the courtesy of Armour and Company.

Ovary: The onset of amenorrhea was at approximately the same time as the first symptoms of hypoadrenalism. The presence of >5 and <50 mouse units of gonadotropin per twenty-four hours in the urine (normal >5 and <100 m.u. per twenty-four hours) (13) on two occasions may be construed as another argument against "panhypopituitarism." The hypothyroidism, hypoadrenalism, and inanition may have been factors in producing the amenorrhea.

Therapy in this case presented two interesting complications. The first was the development of marked generalized edema with the administration of only 2 mg. of desoxycorticosterone acetate daily without supplemental sodium chloride. The edema formation was not associated with manifestations of excessive DCA administration, such as polyuria, polydypsia, and hypertension. It was felt that the edema formation was in some way related to the concomitant thyroid deficiency inasmuch as addition of desiccated thyroid to the therapeutic program permitted the patient to tolerate the same dose of DCA with the development of only minimal edema.

The second complication of interest was the occurrence of two psychotic episodes, when thyroid was first given. The first episode occurred one week after the administration of 8 mg. of thyroid daily. It was amazing to see a patient who formerly had been confined to bed suddenly become very manic and exceedingly strong. She danced about the ward, made her own bed, and helped the nurses with other patients. This lasted approximately five days, and she then resumed a semi-invalid state even though the thyroid administration was maintained. The same psychotic behavior was repeated some three weeks later, four days after the dose was increased to 16 mg. daily. This episode lasted three days and cleared again despite the maintenance of this thyroid dosage. Several weeks later the thyroid dosage was increased to 32 mg. daily and maintained without the occurrence of any further mental disturbance. Means (14) has reported 2 cases of myxedema in which the administration of thyroid was followed by an acute psychosis.

STUDIES WITH GROWTH HORMONE

Introduction

A purified preparation of anterior pituitary growth hormone has been shown by several investigators (15–19) to be diabetogenic in the normal cat and dog, and in the partially pancreatectomized rat. A combination of growth hormone and ACTH will produce diabetes in the normal rat (20) and growth hormone per se increases the glycosuria of alloxanized rats (21). It should be mentioned that in the normal rat (19) and in the acutely depancreatectomized dogs (22) growth hormone may produce an

initial hypoglycemia. Studies in man are few and inconclusive (23, 24).

It seemed of interest to study the effect of growth hormone on carbohydrate metabolism in our patient.

Method

The patient (Fig. 1) was studied under the usual metabolic regimen. The diabetes was controlled by the administration of 24 units of regular insulin daily in divided doses, and the hypoadrenalism by 2 cc. of Lipoadrenal Extract daily plus 3 grams of sodium chloride.

Urine collections were made from 8:00 A.M. to 12 noon, 12 noon to 5:00 P.M., 5:00 P.M. to 9:00 P.M., and 9:00 P.M. to 8:00 A.M. The urine was analyzed for nitrogen and glucose (25). Blood sugar determinations were made ten minutes before each meal and at bedtime (8:00 A.M., 12 noon, 5:00 P.M. and 9:00 P.M.).

The patient was weighed daily and the daily fluid intake and output recorded.

Anterior pituitary growth hormone (Armour)[2] was administered intramuscularly in divided doses as recorded in Table 1.

TABLE 1

Day	Daily dose			Blood glucose (mg.)				Urinary glucose, (Gm./24 hrs.)	Weight, (Kg.)	Fluid balance (ml.)	Daily N balance (Gm.)
	Growth hormone (mg.)	Regular insulin (units)	Lipoadrenal Ext. (ml.)	8:30 A.M.	12:00 N	5:00 P.M.	9:00 P.M.				
1	0	12-6-6	3	234	207	—	228	1.7	46.6	−225	−1.6
2	0	12-6-6	3	247	241	241	224	1.4	47.0	+ 20	+1.0
1	100	12-6-6	3	308	296	220	254	12.3	47.0	− 20	+0.9
2	200	12-6-6	3	299	254	299	254	16.1	47.7	+340	+0.7
3	200	12-6-6	3	315	250	244	234	20.3	49.0	+760	+0.1
4	200	12-6-6	3	Patient vomited					49.7		
1	0	12-6-6	3	168	147	88	93	1.0	48.8	+120	+0.9
2	0	12-6-6	3	171	170	97	140	0.6	48.6	+340	+0.5
1	200	12-6-6	3	177	174	78	135	0.4	48.8	+ 5	+1.0
2	200	12-6-6	3	184	152	134	231	6.3	49.9	+910	+0.8
3	200	12-6-6	3	Patient vomited					50.6		

Results

Results of the administration of growth hormone are shown in Table 1. Listlessness, anorexia, nausea, vomiting, and marked generalized edema developed. The studies had to be interrupted each time because of severe nausea and vomiting. There was no fever at any time. Lewis *et al.* (24) have also noted these untoward side effects following the administration of

[2] Supplied through the courtesy of Armour Laboratories, Lot #J-9501.

growth hormone. Their patients, however, did not show edema. An exact comparison is not possible, as they used a different lot of growth hormone.

As noted, the only definite metabolic change was an increase in urinary glucose. There was no significant increase in blood glucose or in nitrogen excretion.

Comment

The metabolic effects of purified growth hormone in man have not yet been clarified. Until pure growth hormone becomes available for study one may expect confusing results.

Bennett *et al.* (23) have administered purified growth hormone to a girl, judged clinically to be a hypophyseal dwarf. There was no significant alteration in the concentration of blood glucose during two balance studies of twenty days each. The dose varied from 10 to 160 mg. daily. The growth hormone also failed to produce nitrogen storage. Lewis *et al.* (24) have administered purified growth hormone to a dwarf and to a cretin; no changes in carbohydrate metabolism are reported.

Our results are also negative as regards any specific effect on carbohydrate metabolism. The increased glycosuria without significant alteration in the blood sugar level could be due to an increased renal blood flow and/or an alteration in tubular glucose reabsorption. We were not able to carry out renal catheterization studies to establish this point.

The production of glycosuria following growth hormone administration would appear to be significant in the absence of fever. So far as we know, this is the first reported effect of purified growth hormone in man. White *et al.* (26) have presented evidence that growth hormone administered to normal dogs doubled the PAH clearance, almost doubled the inulin clearance and PAH Tm, and also raised the greatly depressed renal function of hypophysectomized dogs to, or above, the normal levels. If this effect occurs in humans it could conceivably explain our results. In view of some experimental evidence that adrenal cortical steroids (27) are necessary for the action of growth hormone, perhaps the lack of effect of growth hormone may have been due to the limited amount of available adrenal steroids.

The occurrence of marked generalized edema during the administration of purified growth hormone is not satisfactorily explained. The lot of growth hormone used was found to contain 1.5 units of the oxytocic and 10 gamma of pressor principle per 100 mg. (Armour assay). This may have been a factor in the fluid retention. To evaluate this factor further, a dose of 2.5 units of posterior pituitary extract was administered every six hours for four doses to this patient. This produced fluid retention, as manifested

by weight gain and decreased urinary volume. No apparent effects were noted after the administration of 1.25 units of posterior pituitary extract every six hours for four doses. It has been suggested (28, 29) that there may be an antagonism between the antidiuretic principle of the posterior pituitary and both the adrenal and thyroid. The presence of hypoadrenalism and hypothyroidism may have made our patient sensitive to small amounts of exogenous posterior pituitary extracts.

FINAL ADMISSION AND POSTMORTEM FINDINGS

The patient was admitted for the fourth and final time because of weakness, lethargy, and generalized edema. The family stated that the "puffiness of the face" had been present for ten days. She had been getting along well until about forty-eight hours before admission, on a daily regimen of thyroid 32 mg., DCA 2 mg., and regular insulin as needed. Twelve hours after admission she had a generalized convulsion and died forty-five minutes later without regaining consciousness.

An autopsy was performed and will be reported in detail at a later date by Dr. Joseph McManus of the Department of Pathology, University of Virginia. We are indebted to Dr. McManus for the report on the findings of pathologic interest in the endocrine system which follow:

Adrenals: There is no adrenal tissue visible grossly. Microscopic examination of both adrenal areas reveals only a thin strip of medulla, no cortex.

Pancreas: There is no abnormality grossly. Microscopic examination reveals no definite abnormality of the islets of Langerhans.

Ovaries: Both ovaries are of normal size grossly. Microscopic examination reveals multiple follicle cysts; no corpus luteum seen.

Thyroid: On section, grossly it is made up of dull, gray, fibrous connective tissue which contains no brown colloid. Microscopic examination reveals complete loss of thyroid structure. The greater part of the gland is made up of a loose myxomatous tissue containing a few sparsely distributed collections of lymphocytes. Remnants of acini can be made out in the center of these collections with the aid of the periodic acid—Schiff's reagent.

Pituitary: The gland appears grossly normal. Microscopic examination reveals approximately normal distribution of the different cell types.

SUMMARY

1. The case history of a female patient with hypoadrenalism, hypothyroidism, secondary amenorrhea and diabetes mellitus, simulating panhypopituitarism but actually due to "end organ failure," has been presented.

2. The administration of growth hormone to this patient produced minimal changes in carbohydrate metabolism. The study was of limited duration because of marked side reactions.

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JCEM

PREGNANEDIOL EXCRETION IN A MASCULINIZING SYNDROME

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IN 1937, Butler and Marrian (1) first called attention to the presence of considerable quantities of pregnanediol contaminating pregnanetriol in the urine of women exhibiting signs of virilization. This phenomenon has been studied in cases of virilism, and high pregnanediol values have been detected in cases which were subsequently proven to be carcinoma of of the adrenal cortex. On only one occasion has excessive pregnanediol been found associated with benign lesions. It has been suggested that the presence of pregnanediol in the urine may be of diagnostic value in indicating the presence of adrenal carcinoma.

We have had the opportunity of studying a young, white female showing obvious signs of virilism with a continual high pregnanediol excretion. Intensive study has suggested that she represents a typical example of the Stein-Leventhal syndrome.

CASE REPORT

History

This patient was first seen in October, 1949, being 18 years of age at that time. It was elicited that there was excessive body hair apparent at about the age of 10 years. The secondary sexual characteristics of the female appeared at about 15 years, although there was no menstruation. At the age of 16 years, the patient received hormones of unknown identity which resulted in six or seven episodes of withdrawal bleeding at intervals of from eight to ten weeks, but this ceased with the termination of therapy. Body and facial hair became more excessive at the age of 17 years and the patient was required to shave daily. She reported that there has never been any libido and although married at the age of 17 years, she has derived no satisfaction from her marital relations.

There were no headaches or visual disturbances. The patient sometimes cried without provocation and had episodes of mild depression. There was no history of allergies of any type. Appetite had always been good, and bowel movements were regular, with two evacuations daily. There was a slight but definite preference for salted foods and the patient reported excessive daily fluid intake.

There were no episodes of dysuria. There was diurnal frequency ($\times 6$) and nocturia ($\times 2$). There had never been any evidence of edema.

The patient had to shave daily and also shaved between the breasts so that she might wear low-neck dresses. She had no specific physical complaints except for the absence of menstruation.

Received for publication August 27, 1951.

Physical examination

The patient was a moderately obese, white female showing evidence of excessive facial and body hair (Figs. 1 and 2). Examination of the head and neck failed to reveal anything abnormal. The thyroid gland was not palpable. The breasts were well developed and free of masses. Heart and lungs were normal to auscultation and the blood pressure, on repeated occasions, averaged about 100/60. The abdomen was rounded,

FIG. 1. Postoperative appearance of patient showing habitus of Cushing's syndrome. There was little regrowth of abdominal hair following surgery.

covered with coarse, black hair, and showed a well developed musculature. The extremities were covered with coarse hair and here also the musculature resembled that of the male rather than of the female.

Pelvic examination revealed a marital introitus with moderate hypertrophy of the clitoris. The cervix was found to be small, in the midline, and with its epithelium intact.

The fundus was anterior, smaller than normal in size, but freely movable. The right ovary seemed to be slightly enlarged and tender to deep palpation. There were no abnormal findings in the left adnexa.

Cyclic estrogen therapy was begun with 3 mg. of synthetic estrogens (Hexesterol) daily, but following two twenty-day courses, withdrawal bleeding was not produced at this dose level. The patient was not seen again until September, 1950.

At this time, there was no change in the clinical history or complaints. The patient

FIG. 2. Patient had refrained from shaving for three days prior to photography.

returned because of an interest in determining the possibility of pregnancy. For this reason, a thorough investigation was made and the following findings, in addition to the history and physical examination, were obtained:

Laboratory data

Hemogram: RBC, 4,020,000; WBC, 5,400; hemoglobin, 13.5 Gm. The Eagle test for syphilis gave negative results. Blood group: A, Rh positive. Urine: appearance, straw and clear; specific gravity 1.008; sugar, 0; albumen, trace; microscopic examination, a few epithelial cells and 6 to 8 WBC per high-power field. Glucose tolerance test: fasting blood sugar, 114 mg.; half an hour after administration of 100 Gm. of glucose, 149 mg.; one hour, 171 mg.; two hours, 141 mg.; and three hours, 118 mg. per cent; no sugar was detected in any of the urine specimens examined and the curve was interpreted as showing a slight but definite decrease in tolerance to glucose, compatible with some increase in adrenal cortical function. The basal metabolic rate was +7 per cent. The concentration of serum sodium was 320 mg. per cent and that of potassium, 18.9 mg. per cent.

The first 24-hour urine specimen, examined on October 18, 1950, showed: volume,

3,180 ml.; 17-ketosteroids, 7.47 mg.; and pregnanediol (determined as free pregnanediol), 18.7 mg. These procedures were repeated on October 27, 1950, with the following results: volume, 3,170 ml.; 17-ketosteroids, 6.3 mg.; and pregnanediol, 20.6 mg.

Examination of the skull was reported by Dr. Aubrey O. Hampton as follows: "Relatively small sella turcica and normal bone. Third molars are unerupted. No overgrowth of mandibles or sinuses and there is no evidence of acromegaly. The reduction in size of the pituitary may be of clinical importance although it is not proof of hypopituitarism."

X-ray examination of the chest revealed the heart and great vessels to be within normal limits and both lung fields were clear.

X-ray examination of the urinary tract (flat film) showed no abnormalities, nor was there any evidence of an adrenal tumor.

In summation, the findings consisted primarily of a normal metabolic rate and 17-ketosteroid excretion associated with a slight but definite decrease in tolerance to glucose and the presence of an abnormal quantity of pregnanediol in the urine. Considering these findings, it was felt that insufficient evidence was present to warrant a definitive diagnosis. Although no definite support could be obtained from the study of the steroid excretion, it was felt that the most likely explanation was a hyperplasia of the adrenal cortex.

Operation

The patient was admitted to the Winchester Memorial Hospital in November, 1950, for exploration by one of us. Prior to laparotomy, a dilatation and curettage was per-

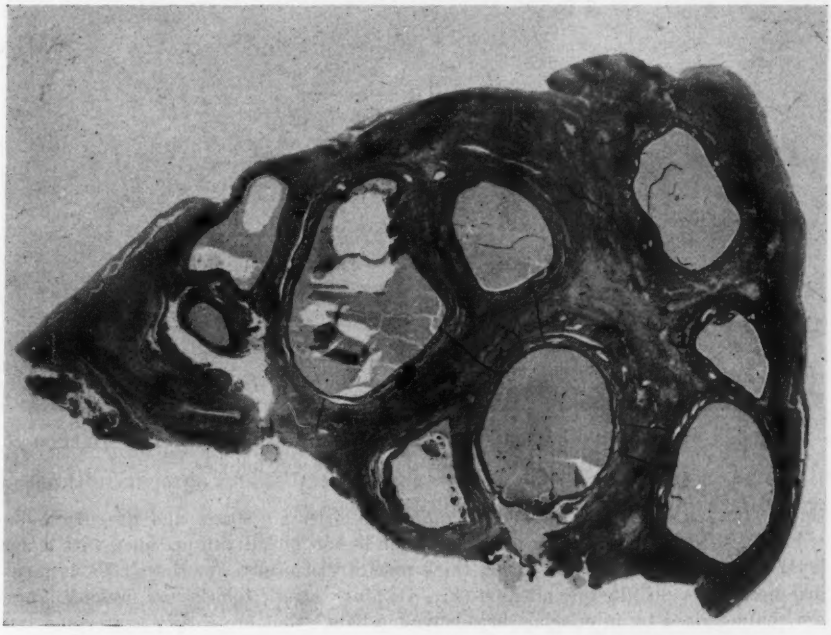

FIG. 3. Small section from right ovary, showing multiple follicular cysts. None measured more than 1 cm. in diameter.

formed but no material was obtained from the uterine cavity which could be submitted for microscopic examination. It was felt that this could be reasonably considered as evidence that an atrophic endometrium existed at the time of surgery.

Through a midline incision, inspection of the pelvic organs revealed a somewhat small uterus, which was otherwise normal. There were no gross changes in either tube. The right ovary was enlarged to the size of a lemon and was approximately twice the size of the left ovary. Each ovary contained small, clear cysts (Fig. 3). The adrenal areas were both manually examined and no gross abnormalities could be detected. A major portion of the right ovary was removed and a biopsy specimen was obtained from the left ovary. There was no evidence of a solid tumor in either gland. The patient made an uneventful recovery from surgery.

Pathologist's report

Microscopic description: "Sections from various parts of the ovary show a disproportionate ratio between the stromal and parenchymatous elements with a decided diminu-

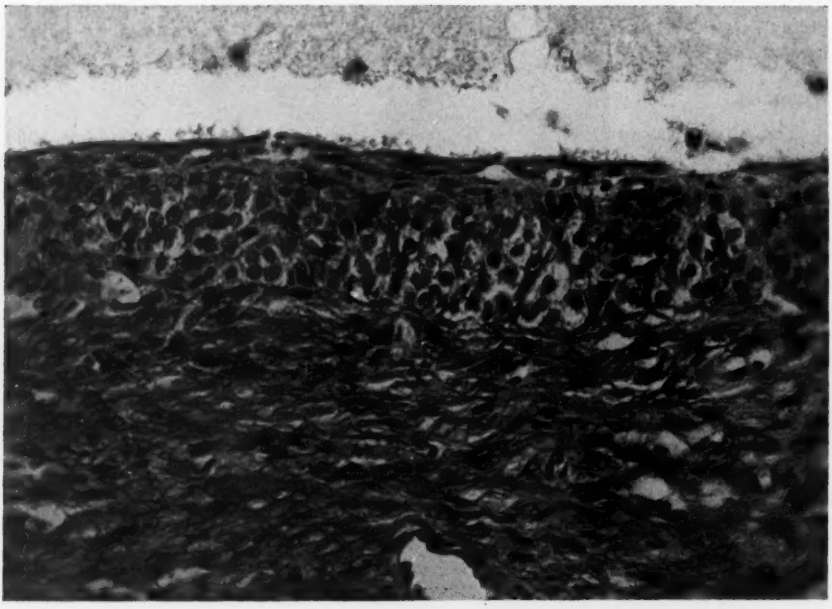

FIG. 4. Cyst wall, showing marked luteinization of theca interna. This situation prevailed in all except the most recent follicles.

tion of the latter components. The primordial follicles are sparse and irregularly distributed. Most of the maturing follicles contain liquor folliculi but are lined with a thin layer of granulosa cells with a rather thick zone of theca cells. The theca cells, in many instances, show definite luteinization (Fig. 4). Many atretic follicles are present. These are characterized by interlacing broad hyaline bands that are closely aggregated into small crenulated masses. Connective tissue fibrils intersperse these masses and in some occupy the central portion showing active vascularization. No structures resembling a

corpus luteum or true corpus albicans are present. None of the cellular components presents a configuration consistent with a neoplasm."

Subsequent course

Following her discharge from the hospital, the patient was again started on cyclic estrogen therapy, 3 mg. of synthetic estrogens daily. She had a menstrual period beginning December 19, 1950, of five days' duration. She also reported the presence of a definite premenstrual prodrome, with the development of breast soreness approximately seven days before the onset of flow. Menstruation continued subsequently in a cyclic manner, approximately every twenty-four days, during treatment with progressively smaller doses of estrogens. The patient reported that it was the opinion of herself and her friends that the hirsutism was decreasing. Inspection of the patient suggested that this was so. A 24-hour urine specimen was submitted on January 24, 1951: volume, 1,800 ml.; 17-ketosteroids, 4.7 mg.; pregnanediol, 0.36 mg. This value for pregnanediol is compatible with the usual findings in this laboratory during the pre-ovulatory phase of the menstrual cycle.

DISCUSSION

In 1935, Stein and Leventhal (2) reported a series of cases of bilateral polycystic ovary associated with amenorrhea. The clinical manifestations associated with this condition were sterility, and a masculine type of hirsutism in 50 per cent of the cases; the findings were less consistent regarding obesity and breast abnormalities. In this and subsequent studies (3, 4, 5), Stein reports that the metabolic rate is usually within normal limits and that the glucose tolerance test and X-ray study of the sella turcica do not reveal any significant alterations from normal. Vaginal smears reveal cells of the postmenopausal type, indicating an absence of normal estrogenic activity, and the endometrial histologic picture varies according to the presence of complete amenorrhea or irregular menses. The identification of bilateral polycystic ovaries at the time of exploration seems to place our case in the group described by Stein and Leventhal. In addition to the characteristic clinical and pathologic manifestations, it was also demonstrated, in this instance, that large quantities of pregnanediol were being excreted in the urine. This is the first time that such an observation has been reported. Stein and Cohen (3) refer to the luteinization of the theca interna of the follicle cysts, but the possible role of these cells as sources of progesterone has not been considered, nor has the effect of continued secretion of large quantities of progesterone been evaluated as a possible etiologic factor in the syndrome. Although pregnanolone (6) and desoxycorticosterone (7, 8, 9) could have served as well as progesterone as the source of the pregnanediol found in the urine, there seems to be sufficient evidence that progesterone is the most likely probability. It is equally accepted that progesterone is also produced by the adrenal cortex (10, 11) but the disappearance of the urinary pregnanediol following the resection

of the ovaries would seem to implicate the ovaries rather than the adrenal as its source.

Previous reports concerning the presence of large quantities of pregnanediol in the urine of women manifesting masculinization attributed to the adrenogenital syndrome have indicated that this finding is closely correlated with the presence of carcinoma of the adrenal cortex. Salmon, Geist, and Salmon (12) studied 7 cases of virilism and found elevated pregnanediol values in the 2 cases subsequently proven to be carcinoma of the adrenal cortex. Venning (13) also reported the presence of increased pregnanediol excretion associated with adrenal cortical carcinoma in one case and benign hyperplasia in another. No corpus luteum was found in these cases at the time of surgery.

The quantity of pregnanediol found in the case reported here would indicate a daily production of progesterone from 180 to 200 mg. This figure is based on findings in this laboratory indicating a consistent conversion rate of 10 per cent in the nonpregnant female in any age group. It is not easy to evaluate the reaction to the continued presence of this quantity of progesterone in the body. There has been considerable controversy regarding the ability of progesterone to inhibit the pituitary content and secretion of gonadotropins. The majority of the data (14, 15, 16, 17) indicate that progesterone possesses the ability to produce a decrease in both pituitary content and secretion of the gonadotropic hormone in rats. Buttner and Trappmann (18) were unable to demonstrate any influence of progesterone on gonadotropic hormone excretion in humans when 20 mg. was administered daily for six days, but it is not felt that this study is conclusive, as the quantity of progesterone was insufficient and the duration of administration too short. Some female patients receiving as much as 250 mg. of progesterone daily have continued to menstruate regularly (19), although it appeared subsequently that their bleeding occurred from an atrophic endometrium (20). Administration at such dosage levels was not continued for a length of time comparable to the clinical situation in our case.

It has been reported that progesterone appears to possess the ability to induce ovulation in the chicken and the monkey. Insofar as the monkey is concerned, Pfeiffer (21) feels that progesterone is the stimulus for the release of luteinizing hormone (LH) from the pituitary, resulting in ovulation; but it appears that this manifestation of progesterone activity depends upon the day of the cycle on which it is administered. Rothchild and Fraps (22) indicate that progesterone will inhibit ovulation in chickens if given at intervals of thirty-six hours or more prior to an expected ovulation. Phillips (23) reported that 1.5 mg. of progesterone inhibited estrous cycles in normal female rats, provided injections were started

three or more days prior to the expected estrus. Similar inhibition in rats has been reported by Selye, Browne, and Collip (24). Makepeace, Weinstein, and Friedman (25) have reported that progesterone inhibits ovulation in the rabbit and this interference, according to this evidence, appears to be exerted at some central site in the ovulatory mechanism rather than through any direct action on the ovarian follicles. Dempsey (26) has demonstrated that when progesterone is administered to guinea pigs, beginning on the tenth day of the cycle, ovulation is inhibited and the ovaries contain normal and atretic follicles of all sizes. Ovulation will occur under these circumstances if LH is administered after the tenth injection of progesterone. The opinion is expressed that the failure of ovulation which follows progesterone administration is caused by inhibition of the release of the LH. It seems a relatively safe assumption that endogenous production of a continuous large amount of progesterone by the theca interna would produce a situation similar to that found following exogenous administration. The presence of many follicles of various sizes and in many stages of regression in our case lends support to the impression of Dempsey that progesterone does not inhibit follicle-stimulating hormone (FSH) production. As the presence of a continuous large quantity of progesterone has been established in this case by the demonstration of large quantities of pregnanediol in the urine, it would appear that the polycystic ovaries resulted from a failure of ovulation induced by the suppression of LH activity of the anterior pituitary.

The explanation for the degree of masculinization is less clear. Although it has been demonstrated that progesterone is capable of converting genetic female frog larvae into males (27), no definite androgenic activity has been ascribed to this steroid. It is of considerable significance, however, that an extremely interesting case of masculinization associated with amenorrhea in a young female has been reported by Twombly (28); his patient also manifested a continuous abnormal excretion rate of pregnanediol glucuronidate. Additional hormone evaluation revealed a slight decrease in tolerance to glucose, normal urinary estrogens, slightly elevated 17-ketosteroids, and a normal metabolic rate. In this instance, a luteoma was found in the ovary, associated with marked decidualization of the endometrial stroma. The latter finding would indicate a somewhat different overall hormonal balance than that in the case reported here; however, the obvious masculinizing features associated with the abnormal progesterone production may indicate some causal relationship. A closely related compound, 17-hydroxyprogesterone, isolated from the cortex of the adrenal gland, has been shown in the castrated rat to possess androgenic function of the order of androsterone, without any progestational activity (29, 30). A reduction compound of 17-hydroxyprogesterone, pregnane-

3,17,20-triol, has been demonstrated by Butler and Marrian (1) in the urine of a woman exhibiting the adrenogenital syndrome with masculinization. This compound was contaminated with quantities of pregnane-3,20-diol (pregnanediol). In view of the masculinization in this and other similar cases, an interesting possibility is raised that a portion of the substance detected in the urine was pregnane-3,17,20-triol. The opinion has been expressed by qualified authorities that the procedure for the isolation of free pregnanediol would lack sufficient specificity to separate these two members of the pregnane series. This possibility will be subjected to further analysis. As 17-hydroxyprogesterone has not been demonstrated in the ovary, its etiologic role in this condition would seem to be minimized.

The possibility of the existence of an alteration in endocrine function resulting in a predominant progesterone activity of the theca interna of the preovulatory follicle must be considered. It is not clear that this assumption would necessitate the conclusion that an estrogen deficiency existed concomitantly, although the theca interna has been mentioned as a source of estrogen. This abnormal progesterone activity of the theca may well result in an inhibition of production and release of LH from the pituitary, resulting in ovulatory failure. This is indirectly supported by the roentgenographic evidence suggesting decrease in pituitary size. FSH production would appear to have continued at a nearly normal rate, as attested to by the many developing follicles. Masculinizing features of the disorder may be a result of a relative increase of normal female androgens or of the presence of a steroid with unsuspected masculinizing properties.

SUMMARY

A case has been presented which appears to be similar to the syndrome described by Stein and Leventhal. In addition to the characteristic clinical and pathologic findings, it was demonstrated that a large quantity of pregnanediol was being excreted in the urine, presupposing an abnormal progesterone production. A possible explanation for the origin of the polycystic ovaries has been advanced.

Acknowledgment

The authors wish to thank Dr. Albert L. Chaney and Dr. Gordon Grant, Director of Research, Ayerst, McKenna & Harrison for valuable information pertinent to the discussion herein presented. Appreciation is also expressed to Dr. E. C. Stuart, Jr., for his kind assistance during the operative procedure.

Addendum

Since the completion of this report, the patient was seen in the office on June 8, 1952. She stated that the date of her last normal menstrual period was February 29, 1952. On June 8 all clinical signs and symptoms of pregnancy were present and the results of a biologic test for pregnancy were reported to be positive.

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MALIGNANT MELANOMA IN A PATIENT WITH OVARIAN AGENESIS. CASE REPORT OF PROLONGED SURVIVAL

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INTRODUCTION

PAST experience notwithstanding, the hope exists that the many mysteries of malignant melanoma may be ultimately unraveled by the blending of the certainty of observation with the uncertainty of imagination. Although it is quite possible that an individual and unique patient with malignant melanoma may become the key to the cause or cure of this disease, yet one must be extremely cautious in deriving probabilities based upon the principles of a single case report. This report is presented because of the prolonged survival of a woman with metastatic malignant melanoma and ovarian agenesis.

It is generally accepted that the course of untreated or metastatic malignancy is one of steady growth, almost always with a fatal termination. A survey of the entire realm of cancer and allied diseases will reveal no tumor more virulent in this respect than malignant melanoma. Although in the natural history of specific neoplastic diseases there may occur occasional periods of spontaneous arrest or even regression, authentic and acceptable cases falling into this category are certainly clinical curiosities. Yet the progressive and autonomous growth of cancer is by no means uniform in every patient. There may occur fluctuations in both the rate and size of growth, due either to extrinsic or intrinsic factors. The recent papers of Shimkin (1), of Dunphy (2) and the classic work of Willis (3) indicate that closely allied to the subject of spontaneous tumor regression is the infrequent but curious and perplexing long-term quiescence of cancer. A proclivity for recrudescence after many years of dormancy is particularly characteristic of malignant melanoma of the eye but is not the typical behavior for similar tumors of the skin or other organs.

There is ample evidence to indicate a close relationship between the sex steroids and the many factors influencing pigmentation in man. The subject has been carefully reviewed by Hamilton (4), who concludes that pigmentation is "regulated by sex hormones to such a material extent that the human skin may be regarded, to some degree, as a 'sex' skin." Pack

Received for publication December 7, 1951.

(5, 6, 7) in several studies has stressed the importance of endocrine activity, notably of the gonads, adrenal cortex and pituitary, upon the clinical characteristics of malignant melanoma, particularly those derived from a pigmented nevus. Malignant melanomata have been observed to occur during the endocrine changes of adolescence with a malevolence seldom seen at this age, ruthless growth with widespread metastases being the rule. On the other hand, Spitz (8) in reporting the experience of Memorial Hospital notes that in 13 cases of juvenile melanomata occurring in childhood prior to the hormonal influences of puberty "only one (7.7 per cent) has had a clinically malignant and fatal course despite the similarity of the juvenile lesions to the malignant melanoma of adults." The incendiary stimulation of malignant melanoma, presumably by the sex hormones, during pregnancy is notorious. Pack (5) reports that he has never seen a patient "who developed a malignant melanoma during pregnancy survive for any length of time." Thus, a relationship between the processes of pigmentation, endocrine activity and the development of malignant melanoma is manifest.

CASE REPORT

M.T. (J.H.H. History No. 172462), a white female, aged 43 years, was seen in the Out-Patient Department of the Johns Hopkins Hospital in March 1947, complaining of a large purple-black mass in the skin of the abdomen of one month's duration. This tumor measured 8×10 cm. and was first noted in January 1947, after the removal of a mole from the patient's left leg. This mole had been burned off with acid in 1946. The patient was noted to be a mentally and physically retarded white female dwarf with extreme kyphosis, who looked much older than her stated age.

Because of her very limited intelligence she was unable to give a coherent history but stated that she had never menstruated. She had had optic neuritis and cystitis at the age of 19, at which time she was found to have no pubic hair. At age 31 she had fractured her left hip and was told that her bones were rarified. Following this she had suffered several fractures of the long bones and at age 37 was again told that her bones were "thin." She reported no intolerance for cold weather. She had entered school at age 8 and quit in the seventh grade at age 15 or 16. The kyphosis had been progressive since the age of 15 and the patient reported that her facial wrinkles and rhagades had been present for about three or four years.

On physical examination the extremities, particularly the upper, were long and the trunk was short. Her arm span was 148 cm. and height 129 cm. There was a tendency to webbing of the neck. The hair was of fine texture, sparse but present in both axillae and the pubic region. There were many light-colored hairy moles over the body. The carrying angle of the arms was normal. She was completely edentulous, the thyroid was small but of normal consistency, there was a very small amount of breast tissue, the lungs were clear, and the heart was not remarkable. Blood pressure was 160/90. The pulsations in the lower extremities were easily palpable. The genitalia were infantile and the vagina was too small to permit examination; neither uterus nor adnexae was palpable on rectal examination. In addition to the abdominal tumor, there were two other masses, one in the left axilla and the other in the left buttock. The lesion in the axilla was removed for

biopsy and proved to be a malignant melanoma. In an effort at replacement estrogen therapy the patient was started on 0.15 mg. of ethinyl estradiol daily, which she took for about three months. The mass in the abdominal wall increased in size.

In October 1947, after a lapse of seven months, the patient again returned because the abdominal mass had grown still larger and appeared ready to ulcerate (Fig. 1). She had omitted medication for four months. In addition to the known masses in the abdominal wall and left buttock there were additional tumors in the left posterior chest-wall,

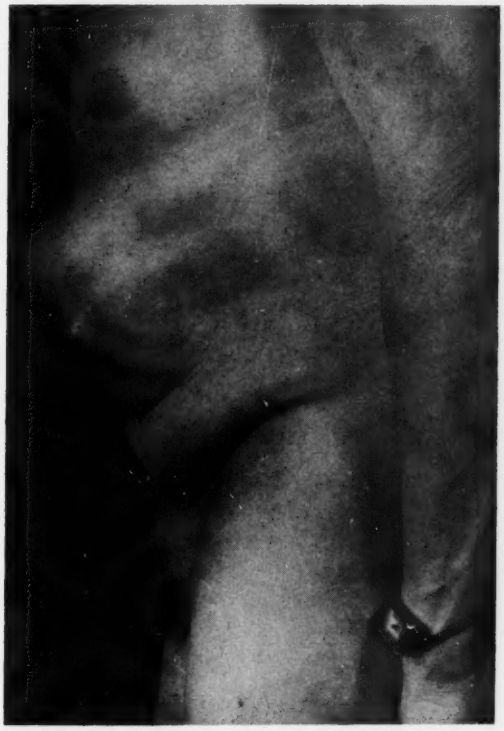

FIG. 1. Photograph taken, October 1947, of the large metastatic malignant melanoma in the abdominal wall, just prior to resection. The undeveloped breasts and sparse pubic hair are apparent.

right axilla and left inguinal region. A palliative resection of the large mass in the abdominal wall was performed on October 24, 1947, and the pathologic examination again revealed malignant melanoma probably metastatic.

The patient then disappeared and was next seen in August 1949, stating that she had never felt better. At this time the findings on general physical examination were unchanged, save that the previously noted masses in the left buttock, posterior thoracic wall and right axilla had *spontaneously disappeared.* The mass in the left inguinal region measured slightly over 1 cm. in diameter. A skull plate revealed a mild degree of hyperostosis frontalis but no evidence of malignant disease.

In October 1949, the patient returned again for follow-up and was found to have glycosuria. A few months later she consented to re-admission to the hospital for regulation of her diabetes and a more careful study of her endocrine status, since cooperation on an out-patient basis had proved impossible. No family history of diabetes could be elicited. There was no change in her general physical examination. The urine showed a 4 plus sugar, but findings were otherwise negative (including tests for melanin). Follicle-stimulating hormone (FSH) studies showed positive results up to 192 mouse units per twenty

FIG. 2. Photograph taken, November 1947, shortly after resection of the abdominal mass. The atrophic skin, facial wrinkles, web-neck, rhagades, kyphosis, undeveloped breasts and sparse pubic hair are characteristic of ovarian agenesis.

four hours, but negative results at 384 mouse units (normal, 6–52 m.u. per twenty-four hours). The fasting blood sugar level was 283 mg. per cent; serum cholesterol, 345 mg. per cent; and NPN 29 mg. per cent. A roentgenogram of the chest revealed marked kyphosis, osteoporosis, distortion of the heart and aorta by the kyphosis, but no evidence of metastases either in the lung fields or in the bony thorax. On January 26, 1950, a biopsy specimen was taken from the left inguinal mass and pathologic examination revealed only scarring and melanin pigment. The patient was discharged on February 5, 1950, taking 20 units of protamine zinc insulin daily.

She was not seen again until she was re-admitted to the Johns Hopkins Hospital on

February 25, 1951. She stated she had been well until three months before admission, when abdominal pain, anorexia and intermittent vomiting developed. On admission she appeared ill, with obvious loss of weight and strength. She was pale, feverish, dehydrated, and vomited intermittently. There was impairment to percussion at both bases; a moderate number of medium moist rales were heard at the right base and a few at the left. The abdomen was flat, soft, non-tender, and without palpable masses or signs of free fluid. Her diabetes appeared regulated but the patient continued to complain of cramp-like abdominal pain, weakness, nausea and vomiting. Her stools showed a 4 plus result with the guaiac test, and roentgenograms again revealed generalized osteoporosis, with now an irregular density in the posterior right mid-lung field. There was a moderate hypochromic microcytic anemia. A gastro-intestinal x-ray series revealed a deformed duodenal bulb without a definite crater. There was a nondescript abnormal small bowel pattern. Her abdomen became distended with signs of fluid. A diagnostic paracentesis revealed 15 cc. of bright red fluid that clotted. On April 21, 1951, a urinary FSH test gave negative results at 96 and 385 mouse units per twenty-four hours. The 24-hour urinary 17-ketosteroid assay was moderately depressed to 2.8 mg. and the estroids were 19.62 gamma by the fluorometric method of Jailer (normal range 5–25 gamma). An exploratory laparotomy was performed on April 25, 1951. In addition to a large amount of cloudy free peritoneal fluid, the entire left upper quadrant of the abdomen was occupied by a dark-colored tumor mass which involved the mesentery, liver and a major portion of both large and small bowel. There were also a number of enlarged lymph nodes, one of which contained black pigment. This was removed for pathologic examination, and a liver biopsy specimen was also obtained. Both biopsy specimens proved on pathologic examination to be malignant melanoma. Examination at the time of the laparotomy revealed the pelvic organs to be infantile. The patient left the hospital on May 4, 1951, and died at home on May 19, 1951. Permission for autopsy was denied.

The period of survival from the onset of grossly evident metastases to death totaled fifty-two months.

DISCUSSION

The syndrome of ovarian agenesis and dwarfism was first described by Turner (9) in 1938. As initially presented, this striking syndrome consisted of decreased stature, sexual infantilism, congenital webbing of the neck and slight cubitus valgus. Since this first report, many investigators have contributed a great deal to the knowledge of this rather clear-cut clinical picture. As pointed out by Wilkins and Fleischmann (10), the developmental defect is one of primary hypo-ovarianism and is the result, in part at least, of an estrogen deficiency. The chest and trunk are of a masculine type and pubic hair is always present. The nipples, breasts, labia, vagina and uterus are infantile. Generalized osteoporosis is usually noted upon skeletal x-ray examination and occasional developmental abnormalities of the osseous system, such as delayed epiphyseal fusion, kyphosis and scoliosis are not uncommon. Other congenital anomalies are reported to be not infrequently associated with this syndrome. When present, they consist of coarctation of the aorta, eye muscle defects, deafness and mental deficiency. The FSH titer is usually elevated but the remaining hormone assays may be within normal limits. The present patient conforms quite

closely to the diagnostic criteria of ovarian agenesis, a condition which appears to be distinctly different from pituitary dwarfism.

Malignant melanomata are considered by Pack and Livingston (6) to be extremely rare in the male or female castrate. However, the present patient was gonad-deficient on a congenital basis. The malignant melanoma, in this particular patient, was characterized by a rather remarkable period of spontaneous regression of multiple metastases and tempered by a protracted period of survival, thus making the subject of this report a case apart and unique in the medical literature.

It has been amply demonstrated that, through hormonal stimulation, both puberty and pregnancy hasten the growth of melanomata. Therefore, by the process of reverse reasoning it has been thought possible to prolong life, inhibit tumor growth and generally to control the ravages of malignant melanomata by castration, hormonal therapy or the production of an endocrine imbalance. As noted by Pack and Schnargel (7) "these clinical experiments have met with uniform failure. We have castrated females and males by irradiation and by bilateral oophorectomy and orchidectomy without perceptibly or measurably influencing the course of the disease." These authors go on to say "we have administered testosterone in maximum doses for prolonged periods of time to women with melanomatosis and have observed no palliative benefits." Howes (11), Herbse (12) and others have reviewed this subject, and the sum and substance of these studies indicate the futility of this therapeutic approach. Yet one must always bear in mind the fact that in most neoplastic diseases, once atypical growth begins, reversibility may be difficult if not impossible to achieve. Factors which may be responsible for initiating cellular insurrection may be only remotely related to the factors responsible for autonomous growth and continued irresistible neoplastic spread. Also, hormones other than the sex steroids (perhaps adrenal or pituitary steroids as yet unknown) may be potent synergists in this sequence of malevolence.

Pituitary irradiation for malignant melanoma has been tried by Pack (7) and his associates, by Wigley and Metz (13) and by others with almost uniformly poor results. Even the most intensive external radiation therapy through rotating ports has not been shown to influence seriously the endocrine behavior or histologic pattern of the normal pituitary. The therapeutic possibilities of adrenal ablation for this "harbinger of death" today remain speculative.

In his Hunterian Lecture, which is an excellent review of the properties and surgical problems of malignant melanoma, Raven (14) points out that the average period of survival is between eighteen months and three years. If at the time of initial operation regional lymph node metastases are found to be present, the gravity of the prognosis is greatly increased. Although

there are no accurate figures for average length of survival after the development of widespread and extensive metastases, a survival period of fifty-two months as observed in the present patient with ovarian agenesis, is indeed noteworthy.

SUMMARY

The unusual course of malignant melanoma occurring in a patient with ovarian agenesis is reported. The relative benignity of the disease in this patient further strongly suggests a hormonal relationship.

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LETTERS TO THE EDITOR

EFFECT OF ASCORBIC ACID ON 17-KETOSTEROID EXCRETION

To the Editor

The rates of excretion of 17-ketosteroids and of formaldehydogenic steroids have been used as indices of adrenal cortical function in scorbutic animals (Clayton *et al.: Brit. M. J.* **2:** 927, 1951; Nadel *et al.: J. Clin. Endocrinol.* **11:** 791, 1951). Findings from this laboratory suggest that this assumption may be fallacious.

We have collected data indicating that the breakdown of cortical hormones in adrenalectomized female rats was diminished by ascorbic acid treatment. It was observed that the hematologic actions of exogenous (cortisone) and of endogenous (induced by ACTH in intact animals) cortical hormones are prolonged by ascorbic acid treatment. This prolongation is associated with a significant decrease in the excretion of 17-ketosteroids in the first 24-hour urine sample following the injections. Adequate controls were run in this series of experiments.

The above observations suggest that the increased 17-ketosteroid excretion in scorbutic animals may be due not to adrenal hyperactivity, as suggested by Clayton *et al.* and by Nadel *et al.*, but rather to an absence of the ascorbic acid. The rate of cortical hormone breakdown in scurvy is probably increased. Other data (McKee *et al.: Endocrinology* **45:** 21, 1949) suggest that the adrenal in scurvy may be hypoactive.

A complete report on the above findings is being prepared for publication.

Habeeb Bacchus, Ph.D.
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ERRATUM

In the Letter to the Editor by Bacchus, Altszuler and Heiffer, entitled "Effect of Ascorbic Acid on 17-Ketosteroid Excretion" (*J. Clin. Endocrinol. & Metab.* **12:** 908 (July) 1952) the authors wish to delete the reference they made to the work of Nadel, since Dr. Nadel did not state, as implied, that he used formaldehydogenic substances as indices of cortical activity.

DIETARY MODIFICATION OF SERUM CHOLESTEROL AND PHOSPHOLIPID LEVELS*

To the Editor:

For more than a generation the matter of relationship between diet and serum lipids, as well as the relationship between diet and the development of clinical atherosclerosis has been a disputed subject. It has been stated and denied that diets high in cholesterol result in significant elevation of the serum cholesterol level in human subjects (1). Recently it has been reported that diets high in fat, even though they may be low in cholesterol, under some circumstances will result in significant elevation of serum cholesterol (2, 3).

In this institution over the past two years, as part of studies in various aspects of lipid metabolism, patients have been maintained on chemically constant formula diets over prolonged periods of time. Some of these diets have been extremely high in fat content. Others have been relatively high in carbohydrate. In some instances, the fat has been derived from vegetable sources; in others, from dairy fat; and in others, from egg yolk. Under the conditions of these studies, the following results have been obtained:

1. All patients receiving carbohydrate-free diets containing large amounts of vegetable fat, usually in conjunction with protein (calcium caseinate), had a major and maintained fall in the concentrations of serum cholesterol and phospholipids, which continued throughout the period of diet administration. A prompt rise to base-line levels occurred when the patient resumed a "usual" mixed diet (Fig. 1). The magnitude of the fall averaged nearly 100 mg./100 cc. for total cholesterol, and approximately 5 mg./100 cc. for serum lipid phosphorus (equivalent to a fall of 125 mg. of lecithin per 100 cc.). Most of the fall in cholesterol is referable to a decrease in the amount of esterified cholesterol. Twelve such patients have been studied in this manner. The study shown in Figure 1 is representative of the group.

2. The addition of as much as 60 grams of cholesterol daily to the diet high in vegetable fat did not result in significant elevation of the serum cholesterol level (Fig. 2). It is possible that the time factor may have been insufficient.

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* This work has been supported in part by grants from the National Institutes of Health and from the Armour Laboratories.

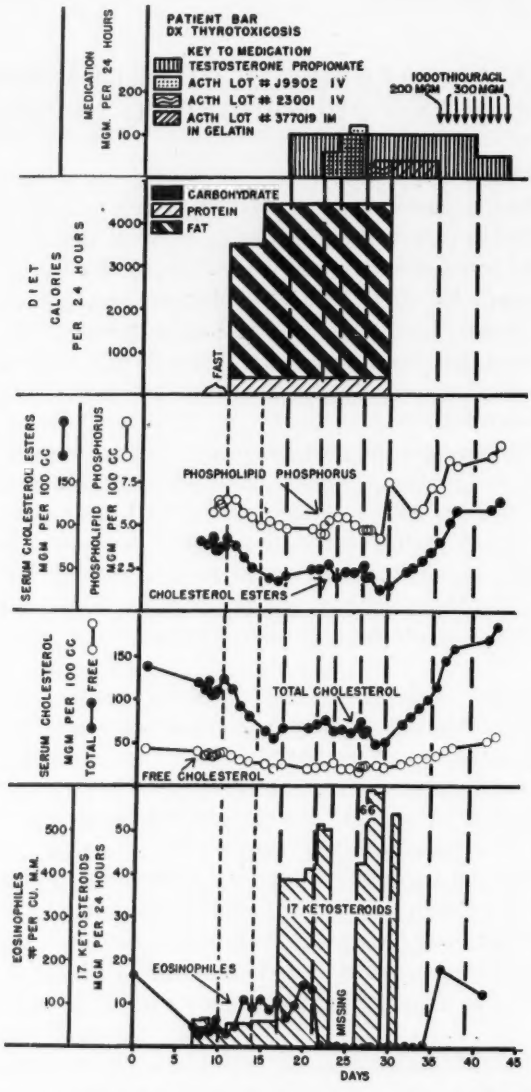

FIG. 1. Showing a major fall in serum cholesterol and phospholipid levels during ingestion of a diet containing very large amounts of vegetable fat. A prompt rise occurred when an average mixed diet was resumed. On the basis of other studies, the hormonal therapy played little or no role in the fall in serum lipids under these experimental conditions.

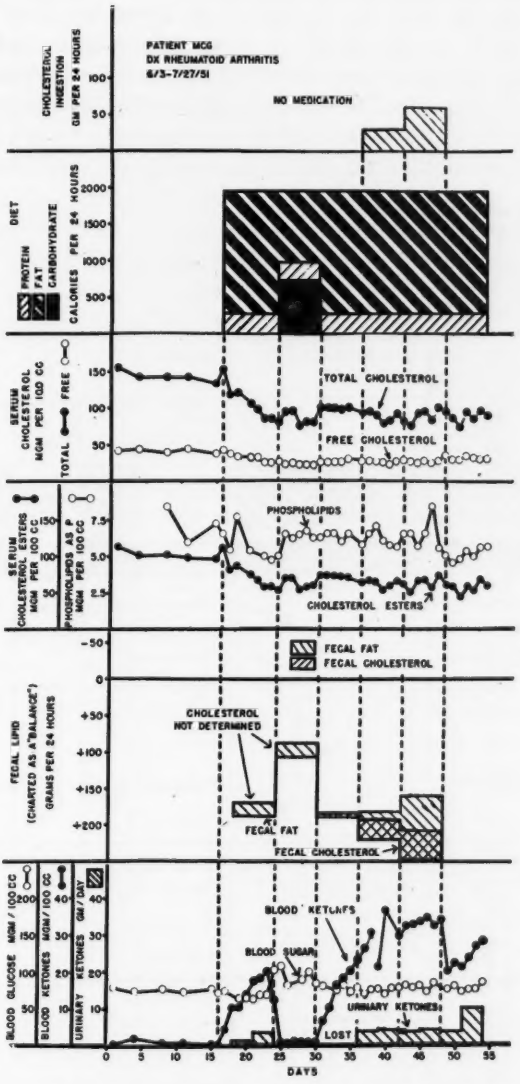

FIG. 2. Despite absorption of large amounts of administered cholesterol, no significant elevation of the serum cholesterol level ensued.

3. Patients maintained on formula diets containing fat of dairy origin or derived from egg yolk, the amount of fat being equal to that administered as vegetable fat, had levels of serum cholesterol and phospholipids essentially identical with those observed in the same patients on an average mixed diet (Fig. 3). The cholesterol content of the egg yolk diet (equivalent to 36 egg yolks) was 9.6 grams per day.

4. Patients maintained on diets containing constant amounts of pro-

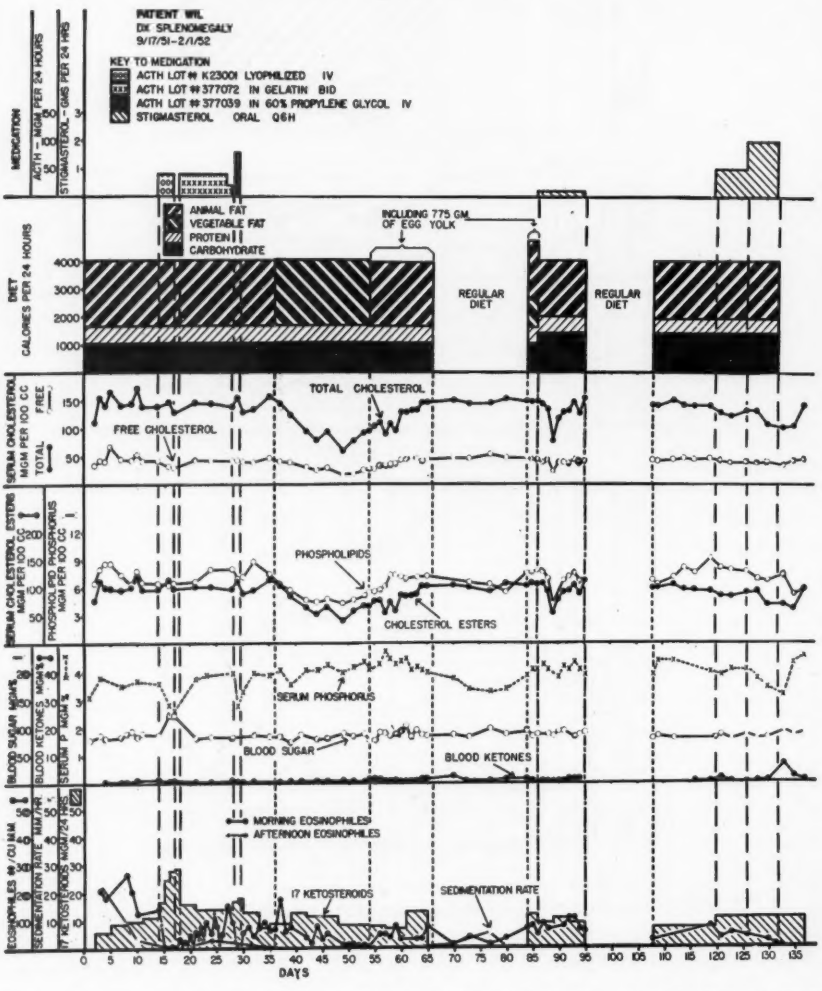

FIG. 3. Relationship of fat of animal and of vegetable origin to the concentration of serum lipids. The changes during stigmasterol administration may be of significance.

tein, but varying proportions of carbohydrate and fat, showed some variability in the levels of serum lipids. These data will be reported later.

From the foregoing observations, it would appear that the ingestion of synthetic diets containing large amounts of vegetable fat consistently results in an impressive fall in the level of serum cholesterol and of phospholipids. Whether this is the result of a positive effect of some material present in the vegetable fat, or attributable to lack of dietary cholesterol, is not yet clearly established. The findings of Peterson *et al.* in chicks (4), and the fall in the serum cholesterol level in one patient during the administration of stigmasterol (Fig. 3), might suggest that the former mechanism was responsible. This is being investigated.

The relationship of the above findings to normal and abnormal cholesterol metabolism in human subjects is unknown at the present time.

Acknowledgment

Grateful acknowledgment is made to Dr. Warren Cox, Mead Johnson and Company, for supplies of Casec; to Dr. Edward Hays and Dr. Grosvenor Bissell of the Armour Laboratories for cerebroside (which was used interchangeably with "Tween 80" as an emulsifying agent) and for supplies of stigmasterol; to Mr. C. D. Pratt for supplies of the emuslifying agent used, namely, polyoxyethylene (20) sorbitan mono-oleate, manufactured and sold by Atlas Powder Company under the trade mark "Tween 80."

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The Endocrine Society

ABSTRACTS OF PAPERS PRESENTED AT THE THIRTY-FOURTH ANNUAL MEETING

THURSDAY, FRIDAY AND SATURDAY,
JUNE 5, 6, AND 7, 1952

Palmer House
Chicago, Illinois

(Abstracts arranged according to numbers on the printed program)

THURSDAY, JUNE 5, 1952

9:30 A.M.: GONADS—REPRODUCTION

CHAIRMEN: ALLAN T. KENYON AND SAMUEL SOSKIN

1. The Bioassay of Pituitary Interstitial Cell Stimulating Hormone (ICSH) in Human Urine: Preliminary Report.

Janet W. McArthur.

From the Gynecological and Children's Medical Services of the Massachusetts General Hospital, Boston, Mass.

A method for the identification and semi-quantitative assay of pituitary interstitial cell stimulating hormone (ICSH) in human urine is described, the weight of the ventral prostate of the hypophysectomized immature male rat being employed as the indicator. The gonadotropins are concentrated by kaolin adsorption and alcoholic precipitation from the ammonium hydroxide eluate, according to the technique of Bradbury, Brown and Brown.

An increment of approximately 100 per cent in prostatic weight was obtained with the equivalent of three hours' output of pooled female urine. Between dosage levels equivalent to three and nine hours' output of such urine, the prostatic weight was roughly proportional to the logarithm of the dose administered.

Clinical application of the method has yielded the following preliminary results: 1) the urine of prepubertal males and females contains no demonstrable ICSH; 2) the urine of postmenopausal women contains ICSH in high concentration; and 3) the urine of normally menstruating women during ovulatory cycles displays maximal ICSH excretion rates in mid-cycle and in the late luteal phase directly before the oncoming menstrual period.

2. Gonadotropin Suppression as a Resultant of the Conversion of Androgen to Estrogen.

C. Alvin Paulsen (introduced by Carl G. Heller).

From the University of Oregon Medical School, Portland, Oregon.

Since 1) it requires far less estrogen than androgen to depress urinary gonadotropins; and since 2) administering testosterone propionate increases estrogen excretion, it is postulated that the efficacy of an androgen in depressing gonadotropins is directly related to the conversion of androgen to estrogen.

To test this supposition three androgens were compared as to their gonadotropin depression effect and the degree to which they cause a rise in estrogens. Seven castrated men were selected for study. Two received testosterone propionate; 3 received methyltestosterone; and 2 received dehydroisoandrosterone, by daily intramuscular injection of 50 mg. of the androgen in oil for six weeks. After a lapse of two weeks a dose of 25 mg. was injected daily for an additional six weeks.

Urinary estrogens and gonadotropins were determined prior to treatment, at two-week intervals during the first course of treatment, and at the end of five weeks of the second course. Neither methyltestosterone nor dehydroisoandrosterone significantly depressed gonadotropins or altered estrogen excretion. Testosterone propionate, however, depressed gonadotropins to zero and increased estrogen excretion approximately twenty-fold.

These findings support the suggestion that gonadotropin suppression is a resultant of androgen-to-estrogen conversion.

3. Gynecomastia and Retarded Sexual Development Resulting from a Long-Standing Estrogen-Secreting Adrenal Tumor.

Richard L. Landau, Dwight E. Clark (by invitation) and *Benjamin Stimmel.*

From the Departments of Medicine and Surgery, University of Chicago and the Rees-Stealy Clinic Research Foundation, San Diego, California.

A benign adrenal tumor produced a moderate nonprogressive gynecomastia which began with puberty at the age of 13, slight retardation in secondary sex characteristics and suppression of spermatogenesis in a 28-year-old man. The excessive secretion of estrogen by the tumor thus resulted in a syndrome which might readily be confused with pubertal gynecomastia or with testicular defects and gynecomastia of different etiology.

The possibility of an adrenal tumor in this case was suggested by the high normal excretion of 17-ketosteroids (25 mg. daily) with heightened (15 mg.) Pettenkofer chromogens, a measure of dehydroisoandrosterone and related steroids. Urinary estrogens were ten times the normal value for men (86γ estrone, 15γ estradiol and 57γ estriol, with simple hydrolysis; after Zn-HCl hydrolysis, 8γ estrone, 114γ estradiol and 47γ estriol). No other signs of adrenal dysfunction were elicited. Formaldehydogenic steroid excretion was .94 mg. per day, a normal value. Several ejaculates contained about 10,000,000 preponderantly abnormal spermatozoa per ml.

Following removal of the tumor, urinary estrogens dropped to normal, 17-ketosteroids to 8 mg. and Pettenkofer chromogens to 0.8 mg.; normal ejaculates were secured, and sexual maturation again advanced. Estrogen-secreting as well as other functioning adrenal cortical tumors may be associated with increased dehydroisoandrosterone excretion.

4. The Isolation and Identification of Testosterone, Δ^4-Androstenedione-3,17, and 7-Ketocholesterol from Spermatic Vein Blood.

C. D. West (by invitation), *V. P. Hollander* (by invitation), *T. H. Kritchevsky* (by invitation) and *K. Dobriner.*

From the Sloan-Kettering Institute for Cancer Research, New York, N. Y.

Blood obtained by cannulating the spermatic veins of anesthetized, heparinized male dogs was analyzed for its steroid content. The serum from a total of 6,050 cc. of whole blood obtained from 6 dogs was extracted with ether. The neutral ether-soluble fraction was partitioned between hexane and 70 per cent ethanol. The 70 per cent ethanol fraction was subjected to Girard separation. The ketonic fraction was further purified by chro

matography on silica gel and alumina. Testosterone, Δ^4-androstenedione-3-17, and 7-ketocholesterol were isolated and identified by infrared analysis and paper chromatography. Cholesterol and other as yet unidentified compounds were also obtained.

5. The Use of Spleen Glucuronidase as a Hydrolytic Agent for Pregnanediol Assays.

Saul L. Cohen and *M. M. Goldfine* (introduced by G. T. Evans).

From the Department of Physiological Chemistry, University of Minnesota Medical School, Minneapolis, Minn.

Although preparations of spleen glucuronidase effectively hydrolyze sodium pregnanediol glucuronidate (Cohen: *J. Biol. Chem.* **192:** 147, 1951) the liberated pregnanediol cannot be colorimetrically assayed unless the hydrolysate is first partially purified. Using the purification and colorimetric assay techniques of Sommerville *et al.* (*Lancet* **2:** 50, 1949), it has been found that pregnanediol glucuronide is rapidly and quantitatively hydrolyzed by spleen glucuronidase concentrate. Pregnanediol conjugate of pregnancy urine in amounts as low as 100 μg is similarly quantitatively recovered. Since no additional pregnanediol could be found in human pregnancy urines hydrolyzed by acid after the glucuronidase hydrolysis, it would thus seem that all of the pregnanediol in such urine is conjugated as the glucuronide. Application of these techniques to urine specimens obtained during various phases of the menstrual cycle have yielded white crystalline residues with melting points only 2–15° lower than that of pure pregnanediol. The use of this glucuronidase hydrolysate readily permits pregnanediol assays on urines from even the early follicular phase of the menstrual cycle.

6. The Isolation of Progesterone from Human Placenta.

William H. Pearlman and *Emily Cerceo* (by invitation).

From the Department of Biochemistry, Jefferson Medical College, Philadelphia, Pa.

The authors (*J. Biol. Chem.* **194:** 807, 1952) recently isolated allopregnanol-3β-one-20, pregnanediol 3oC,20oC and allopregnanediol-3β, 20oC from an acetone extract of human placenta. They wish now to report the isolation of progesterone in good yield from an ether extract of another batch of full term placenta; the hormone is present in amounts of 1 to 2 mg. per kilo of tissue and appears to be the major oC,β unsaturated ketone present. Procedures are described for the precise estimation of the progesterone content of extracts of individual placenta, based on ultraviolet spectroscopic examination of purified ketonic fractions.

7. High Progestational Activity of 19-norprogesterone.

W. W. Tullner (by invitation) and *Roy Hertz.*

From the National Institutes of Health, Bethesda, Maryland.

In the course of evaluating a series of pregnane derivatives for clinical potentialities, the physiologic activity of 19-norprogesterone was compared with that of progesterone. The former has been reported to be "fully as active as progesterone, perhaps even more so" (Allen and Ehrenstein: *Science* **100:** 251, 1944). Material prepared by Ehrenstein was a resinous substance believed to be a mixture of stereoisomers. The 19-norprogesterone used in this study was the pure crystalline compound, m.p. 144–145°, synthesized by Miramontes, Rosenkranz, and Djerassi (*J. Am. Chem. Soc.* **73:** 3541, 1951). Results of several series, employing the Clauberg rabbit test for endometrial proliferation, were:

250 mcgm. of progesterone, +1, 19-norprogesterone, +4 (full proliferation); 125 mcgm. of progesterone, negative, 19-norprogesterone, +4. With the Corner-Allen method of bioassay, 500 mcgm. of progesterone produced endometrial proliferation comparable to 62.5 mcgm. of 19-norprogesterone (± to +3), whereas 125 mcgm. of 19-norprogesterone repeatedly evoked full proliferation. These findings indicate an activity for 19-norprogesterone 4 to 8 times greater than that of progesterone.

8. New Ways to Increase Progesterone Activity.

Paul Engel, Carlos M. Garcia V., Siegfried Fischl and *Aldo Muggia* (introduced by Max Goldzieher).

From the Research Institute of Life Laboratories and The Medical Department of the Seguro Social, Quito, Ecuador.

After previous investigations which proved that colloid vehicles increase the activity of estrogens and show some properties of degradated pectin, we prepared, by means of an innocuous synthetic oily compound with hydrophile groups, hydrosoluble progesterone with degradated pectin. The activity of this preparation was studied (Corner test) and compared with progesterone in oily solution, progesterone microcrystals in aqueous suspension, progesterone micocrystals suspended in degradated pectin solution, and, hydrosoluble progesterone in aqueous solution without degradated pectin. All preparations were administered, in doses of 4.25 to 1 mg. subcutaneously and, when possible, orally, intravenously and intraportally. Degradated pectin protects the hormone against its inactivation by the liver and probably also by digestive juices. Degradated pectin increases considerably the biologic action of hydrosoluble progesterone and that of microcrystals. Hydrosoluble progesterone with degradated pectin, given orally, is significantly efficient, *i.e.*, much more active than equal doses of parenterally administered oily solutions, and practically as effective as equal doses of parenterally administered microcrystalline suspension in degradated pectin. Hydrosoluble progesterone with degradated pectin, administered orally in daily doses of 5–10 mg. of progesterone to 25 patients with progesterone deficiency (hypoluteinic dysmenorrhea, functional menometrorrhagia, imminent and habitual abortion, etc.) gave highly satisfactory results.

9. Serum and Urinary β-Glucuronidase Levels in Patients with Prostatic Cancer.

F. T. Brayer (by invitation) and *J. B. Trunnell.*

From the University of Texas, M. D. Anderson Hospital for Cancer Research, Houston, Texas.

Daily measurements of serum and urinary β-glucuronidase levels have been made in 14 patients with prostatic cancer. Individual periods of investigation ranged from two to eight months, during which many other measurements were made with equal frequency; chief among these were serum acid and alkaline phosphatase and urinary steroid metabolites. Responses to castration, ethinyl estradiol, progesterone, hypophyseal irradiation, *a*-methopterin, 2,6:diaminopurine, folinic acid, and glucuronic acid were studied. Clinical benefit was consistently seen to be closely related to an abrupt increase in the output of β-glucuronidase in the urine. Exceptions were seen when 2,6:diaminopurine was administered. Under the latter circumstances there was evidence of clinical deterioration, in connection with a "hump" in the curve representing β-glucuronidase excretion.

Folinic acid and glucuronic acid (lactone) administration were associated with decreased urinary excretion of the enzyme, lactone causing an immediate and striking depression thereof. For the most part the serum levels of β-glucuronidase were affected to a

much smaller degree than were the urinary levels. Although no satisfactory explanation for the observed phenomena is at hand, it appears that the enzyme studied plays a highly significant role in the control and relapse of prostatic cancer.

10. The Production of Hypertension, Nephrosclerosis and Cardiac Lesions by Methylandrostenediol (17α-Methyl-Δ^5-androstene-3β,17β-diol) Treatment in the Rat.

Floyd R. Skelton (introduced by G. A. Grant).

From the Research and Biological Laboratories, Ayerst McKenna & Harrison Ltd., Montreal, Canada.

Methylandrostenediol (MAD) is a steroid which has been reported to possess androgenic, estrogenic, spermatogenic, anesthetic, progestational, renotropic and protein-anabolic properties. No appreciable corticoid activity has been found in the experimental animals, although sodium retention has been shown to accompany the nitrogen retention in the human. Preliminary experiments suggested that this steroid, rather than antagonizing desoxycorticosterone acetate (DCA) in the rat, produced very similar changes.

A more detailed experiment was thus performed in which MAD and DCA in daily doses of 10 mg. and 2 mg., respectively, were injected subcutaneously for six weeks into female, hooded rats sensitized by unilateral nephrectomy and 1 per cent NaCl in the drinking water. During that time the saline intake and blood pressure of both MAD and DCA-treated rats progressively increased, and at autopsy marked cardiac and renal enlargement were observed. The adrenals of the MAD group appeared hemorrhagic, and the kidneys were pale with numerous hemorrhagic spots over the surface. Pale, white nodules were grossly visible in the hearts of both treated groups. Microscopically there were nephrosclerosis and glomerular degeneration in the kidneys, and "hyalinosis" nodules scattered throughout the hearts of rats treated with both DCA and MAD. The significance of these effects of a C19 steroid is discussed.

11. Clinical, Hormonal and Histologic Study of Human Brucellosis Orchiepididymitis.

F. A. de la Balze, R. E. Mancini (by invitation), *G. Iacapraro* (by invitation), *F. Arrillaga* (by invitation) and *E. A. Molinelli* (by invitation).

III Catedra de Clinica Medica de la Facultad de Medicina, Servicio de Urologia del Policlinico Pirovano y Seccion Estudios Epidemiogicos del Instituto Malbran, Ministerio de Salud Publica de la Nacion, Buenos Aires, Argentina.

The testes and epididymes of 8 brucellosis patients ranging between 27 and 50 years of age, in whom *Brucella* had been isolated by blood culture, were studied from the anatomic and functional point of view. Seven presented clinical orchiepididymitis, which was unilateral in 5 instances and bilateral in 2.

Biopsy of the testes was performed between eleven days and seven years after clinical onset of orchiepididymitis. Bilateral biopsy was performed in 6 patients, and unilateral in 1. The remaining specimen was material from autopsy, death having occurred after thirty-eight days from ataxoadynamic brucellosis without clinical damage of external genitals.

The 14 testes showed: 1) Acute orchitis in one. 2) Sclerotic lesions in 13, peritubular focal in 11, peri and intertubular focal in 5, and diffuse peri and intertubular in two. 3) Sclerotic lesions associated with granuloma in two. 4) Sclerotic testes with hyaline sclerosis of the intima of the arteriola. 5) Germinal epithelium with atrophy of varying degree, the intensity being parallel to the peritubular sclerosis. 6) Absence of Leydig cells in testes with diffuse sclerosis, and in foci of intertubular sclerosis.

Histologic study of the epididymis was made in 2 patients. One had acute diffuse epididymitis and the other chronic diffuse epididymitis.

Spermograms of 7 patients showed: azoospermia in 3, oligosteratospermia in 2 and a normal spermatic pattern in 2. Excretion of 17-ketosteroids was diminished in 3 patients with active brucellosis, and was normal in 2 convalescents. Gonadotropin excretion was augmented in 2 cases and was normal in 5.

In some patients histologic examination revealed sclerosis in the testes when there had been no clinical symptoms of orchiepididymitis. A divergence between clinical symptoms and histologic lesions was evidenced.

From the authors' experience, male sterility may result from brucellosis.

12. The Diagnostic Value of Pneumoperitoneum in Sex Endocrine Disorders.

H. S. Guterman, R. H. Kunstadter and *A. S. Tulsky* (by invitation).

From the Department of Metabolic and Endocrine Research, Medical Research Institute; the Department of Pediatrics, Sarah Morris Hospital for Children; and the Department of Obstetrics and Gynecology, Michael Reese Hospital, Chicago, Illinois.

In some sex endocrine disturbances detailed physical examination as well as laboratory studies may be diagnostically inconclusive. In such cases transabdominal pneumoperitoneum may aid in establishing the clinical diagnosis and thus avoid laparotomy. The method is of special value in the study of children with such disturbances, because of the difficulty of adequate pelvic examination. In addition, it may be necessary to make an early diagnosis for future management as well as prevention of further emotional and physical disturbances.

The technique consists of introducing a measured amount of CO_2 into the peritoneal cavity and then visualizing radiographically the outline of the pelvic viscera.

Cases which include ovarian agenesis, precocious puberty, hypopituitarism, cystic ovaries and intersexuality, have been studied by this technique and are discussed.

13. Transvaginal Peritoneoscopy (Culdoscopy of Decker)—A New Instrumentality for Evaluation of Ovarian Function. II. Evaluation and Management of Secondary Amenorrhea.

A. R. Abarbanel.

From the Department of Obstetrics and Gynecology, College of Medical Evangelists, Los Angeles, California.

Twenty-five women with secondary amenorrhea of six months' to several years' duration were peritoneoscoped transvaginally as a simple office procedure (Abarbanel, A. R.: *Urol. & Cutan. Rev.* **55**: 339–344, 1950). On the basis of the clinical picture, including basal body temperature curves, endometrial biopsies and vaginal smears, the gross anatomic findings were correlated with therapeutic responses to various gonadotropins, combinations of steroids, and so-called "stimulating" x-ray to the pituitary and/or ovaries, as well as bilateral ovarian resection. Several women were also peritoneoscoped following therapy.

Accordingly, three broad groups have been found: *Group I—Primary follicular failure* —Two types of ovaries are visualized—one is the small, crenated almond-shaped ovary; the other is the small, rounded gonad with a smooth, thick, whitish fibrotic capsule. *Group II—Multiple parvi-cystic ovaries*—The average sized gonad contains many tiny cystic follicles resembling a small gherkin pickle. *Group III—Large polycystic "oyster"*

ovaries—including the Stein-Leventhal syndrome. Here the gonad is usually 2 to 3 times the average in size. On the surface may be seen several follicles about 0.5 to 1 cm. in diameter, or occasionally larger ones. The capsules may be thin and translucent, or thick, whitish and fibrotic.

In review, transvaginal peritoneoscopy is a very valuable diagnostic procedure, "like an exploratory laparotomy in the office," in evaluating ovarian function, particularly when correlated with other clinical data. On the basis of the visual findings, the prognosis may be more accurately determined.

2:00 P.M.: ACTH AND ADRENALS

CHAIRMEN: IRVINE MCQUARRIE AND CURT RICHTER

14. The Inactivation of ACTH in Mammalian Blood.

Gregory Pincus, Oscar Hechter and *Thomas Hopkins* (by invitation).

From the Worcester Foundation for Experimental Biology, Shrewsbury, Mass.

Certain highly purified ACTH preparations added to bovine whole blood or plasma maintain full potency over a period of several hours. When these same preparations are added to rat or human blood or plasma 40–70 per cent of the activity is lost in one hour. Heating the rat or human blood at 50°C. for from three to four hours prevents the inactivation observed in unheated blood. When these same preparations are added to a mixture of beef blood (which fails to inactivate) and heated rat blood (which also fails to inactivate) one again observes 40–70 per cent inactivation in one hour of incubation. These data suggest the presence of an ACTH inactivating system consisting of a heat labile and a heat stable substance. Both factors are present in rat and human blood, but the heat stable factor appears to be lacking in bovine blood. Further verification is obtained with the study of certain ACTH preparations which contain the heat stable factor as part of the total extraction. These ACTH preparations when added to bovine blood or plasma lose 50–80 per cent of their ACTH activity after one hour's incubation. Data indicating variations in the ACTH inactivating capacity of blood from various human subjects are presented.

15. Blood ACTH in Rat and Man.

Katherine L. Sydnor and *George Sayers.*

From the Department of Pharmacology, University of Utah College of Medicine, Salt Lake City, Utah.

The oxycellulose technic of Astwood for the isolation of pituitary ACTH has been successfully applied to the quantitative analysis of the hormone in blood. Blood is mixed with glacial acetic acid and the mixture heated for thirty minutes at 70°C; the acetic acid is diluted to 1.0 N with water. Oxycellulose is added and the mixture stirred for twenty-four hours. The oxycellulose is collected and the ACTH eluted with 0.1 N HCl Eluates are assayed by the adrenal ascorbic acid-depletion technic in hypophysectomized rats. Blood from hypophysectomized rate induces no detectable response (dose of eluate equivalent to 18 ml. of blood). ACTH added to blood from hypophysectomized rats is quantitatively recovered. Estimates of ACTH per 100 ml. blood in experimental animals are as follows: from decapitated normal rats, less than 1.1 milliunit (one milliunit equals one thousandth of a U.S.P. unit); from normal rats acutely exsanguinated, 2.5 ± 0.5 milliunits; from adrenalectomized rats (seven days postoperatively), 11.1 ± 2.0 milliunits. The value for adrenalectomized rats, obtained by direct transfusion of adrenalectomized

rat blood into assay animals (15.7 ± 2.0), is in satisfactory agreement with the value obtained by the oxycellulose technic. Preliminary human studies (2 normal subjects and 3 patients with Addison's disease) indicate that blood from normal adult males contains less than one milliunit per 100 ml., whereas blood from male patients with Addison's disease contains 2 to 4 milliunits per 100 ml.

16. Rare Dwarfism with Chronic Hypoglycemia and Convulsions: Observations on ACTH and Thyroid.

Matthew M. W. Steiner (introduced by Lawson Wilkins).

From the Children's Memorial Hospital, Chicago, Illinois.

Observations were made on 2 siblings, a male aged 12 years and a female aged 5 years, whose heights were 28 inches and 23 inches, weights 20 pounds and 13 pounds, and bone ages 6 months and 3 months, respectively. Body habitus was that of athyreotic cretins with mental development roughly consistent with bone age.

Onset of hypoglycemic episodes ranged from birth to 5 months of age. There was no uptake of radioactive iodine over the thyroid region. Extreme sensitivity to oral thyroid was exhibited repeatedly; as little as 1/16 gr. daily for four days produced marked weight loss and hypoglycemia.

ACTH (15 mg., four times daily) resulted in fasting blood glucose values of 94–115 mg. per cent. Subsequently a dose of $2\frac{1}{2}$ mg. once every four or five days was sufficient to maintain a fasting level of 52–72 mg. per cent and permitted tolerance to initial thyroid medication. Rapid increase in the dose of thyroid to 1 gr. daily necessitated an increase of ACTH to 15 mg. every four to seven days in order to maintain values above 45 mg. per cent. Clinical improvement in patients was characterized by increased appetite, color and activity, loss of constipation, growth of scalp hair, and decreased size of the tongue.

The etiology of this syndrome remains obscure.

17. Alterations in 17-Hydroxycorticosteroids of Peripheral Blood Following Acute Whole-Body Irradiation of the Dog.

J. Z. Bowers, D. H. Nelson (by invitation), *R. Bay* (by invitation) and *L. T. Samuels.*

From the Radiobiology Laboratory and Department of Biochemistry, University of Utah College of Medicine, Salt Lake City, Utah.

Acute whole-body irradiation of the dog at an L.D.50 level produces a pronounced immediate increase in the 17-hydroxycorticosteroids of the blood as determined by the chromatographic method of Nelson and Samuels. The increase, which is detected as soon as irradiation ceases, persists for approximately twenty-four hours and is followed by a fall to normal levels with a preterminal rise.

These findings are the first direct measurement of changes in adrenal cortical function after acute wholebody irradiation and clarify, in part, previous reports regarding the role of the adrenal cortex in acute irradiation injury.

The promptness with which the increase in blood levels appears emphasizes the speed of discharge of the pituitary-adrenal system and suggests therefore the possibility of a direct "stimulating" effect of irradiation on this system, or indirect stimulation through humoral factors. The pattern of the blood steroids does not follow that described in existing information on histopathologic changes in tissue after acute whole-body irradiation, but does parallel the anorexia, vomiting and lassitude which occur immediately after irradiation.

18. Clinical Studies on Eosinophil Rhythm.

Edmund B. Flink and *Franz Halberg* (introduced by Irvine McQuarrie).

From the Departments of Internal Medicine and Physiology, University of Minnesota, Minneapolis, Minn.

A change in the number of eosinophils during a period from 6:30 A.M. to 9:30 A.M. is the index employed in testing for endogenous eosinopenia. A decrease of 20 per cent or more is defined as a negative result. Negative results were obtained in 68 per cent of 200 tests on patients without adrenal-cortical insufficiency.

Forty of 41 tests on 20 patients with pituitary and/or adrenal-cortical insufficiency gave positive results. This group included 10 patients with Addison's disease (greatest decrease: −16 per cent; mean change: +4 per cent), 5 bilaterally adrenalectomized patients (greatest decrease: −18 per cent; mean change: +3 per cent), and 5 patients with panhypopituitarism (mean change: +12 per cent; two decreases: −8 per cent and −23 per cent). Negative results with the endogenous eosinopenia test appear to be strong evidence against pituitary and/or adrenal-cortical insufficiency.

The 24-hour eosinophil rhythm was studied by counts on samples of venous blood withdrawn at ninety-minute intervals. In contrast to the limited random variation of eosinophils in adrenal-cortical insufficiency and to the markedly reduced rhythm in untreated hypothyroidism, hospitalized patients with adequate adrenal-cortical function present a rhythm comparable to that of normal subjects.

19. Hormonal Influences Upon the Distribution and Fate of the Eosinophilic Leukocyte.

Jacques Padawer (by invitation) and *Albert S. Gordon.*

From the Department of Biology, Washington Square College of Arts and Science, New York University, New York, N. Y.

Eosinophilic leukocytes were found in high concentrations in the peritoneal and pleural fluids of a modified Long-Evans strain of normal rats. Subcutaneous injections of cortisone led to reductions in total eosinophilic cell numbers, both peripherally and peritoneally, in normal and splenectomized animals. Reductions in percentages of eosinophils occurred to a similar extent in peripheral blood and in the peritoneal fluid of normal rats under the influence of cortisone. Neutrophilic polymorphonuclear leukocytes, although not encountered normally in peritoneal fluid, did appear following cortisone, but not after epinephrine or exposure to lowered barometric pressure. All three agencies caused eosinophilic cell breakdown, accompanied by karyorrhexis and pronounced shrinkage of the cell, a phenomenon seen most clearly in peritoneal fluid. Peritoneal and pleural macrophages were observed containing engulfed eosinophilic elements. In the peritoneum, the numbers of macrophages containing eosinophilic elements were directly proportional to the numbers of shrunken eosinophils. Some uptake also occurred in the control untreated rats, suggesting a possible mechanism for disposal of eosinophilic leukocytes under normal conditions. Epinephrine, cortisone and lowered barometric pressures all increased the numbers of peritoneal macrophages containing phagocytized eosinophilic elements.

20. An Experimental Adrenogenital Syndrome in the Frog.

Emil Witschi.

From the State University of Iowa, Iowa City, Iowa.

The administration of relatively high dosages of estradiol (1 mg./liter) to larval frogs, starting at the time when the adrenal begins to organize, produces a conspicuously hy-

perplastic development of the cortical tissue. The histologic differentiation is premature, but mostly, the total volume of the gland is increased tenfold and more. At the time of metamorphosis, one still finds a high mitotic activity of the cortical cells. However, the reduced size of the mesonephric bodies suggests that to some extent, presumptive mesonephric blastema may have differentiated into corticoadrenal lobules. It is now of particular interest that 1) low doses of estradiol, *e.g.*, 0.05 mg./liter, produce a complete masculinization of all female larvae (*e.g., Rana temporaria*, controls: 24 males+39 females; experimental: 37 males). Intermediate series show that the moderate enlargement of the adrenal causes retardation of ovarian development and various degrees of intersexuality. The bearing of the results on the possible interpretation of certain known features of normal and pathologic adrenal development in man is discussed.

21. Some Factors Affecting the Success of Pharmacologic Blockade of Ovulation in Rats.

John W. Everett.

From the Department of Anatomy, Duke University School of Medicine, Durham, N. C.

Under controlled lighting conditions (fourteen hours of light per day, regulated by time-switch) the activation of the pituitary leading to ovulation takes place predictably in our 4-day cycle rats within restricted limits soon after 2:00 P.M. on the day of proestrus. Nembutal (30 mg./Kg., intraperitoneally) injected at that hour, or phenobarbital (75 mg./Kg., subcutaneously) injected at 1:00 P.M. will regularly block this activation (Everett and Sawyer: *Endocrinology* **47:** 198, 1950). Ovulation may be blocked day after day by injection at the same hour. In 5-day cyclic rats, however, to accomplish predictable blockade with barbiturates it has been found necessary to prolong the action of even the "long-acting" phenobarbital, by a supplementary injection (50 mg./Kg.) at 4:00 P.M. Apparently the "critical period" during which pituitary activation may occur among various 5-day cyclic rats extends for several hours longer than among 4-day cyclic rats of the same strain.

Dependence of the timing of pituitary activation on the lighting rhythm is shown by the results of advancing the time-switch three hours. During the next two weeks, repeated attempts at blockade in 4-day cyclic rats at the usual clock hour became progressively less effective, but again became regularly effective within two weeks after returning the time-switch to normal setting. By inference: erratic lighting would foster erratic ovulation time. On the other hand, no significant effect of erratic room temperature fluctuation has been recognized.

Genetic uniformity and prepuberal conditioning to the lighting regimen may both be of some importance, but these cannot be evaluated at present.

22. Studies on Pigmentation in Addison's Disease and Following ACTH and Cortisone Administration.

Brian H. McCracken (by invitation) and *Thomas C. Hall* (by invitation). (Introduced by George W. Thorn).

From the Department of Medicine, Harvard Medical School; the Medical Clinic, Peter Bent Brigham Hospital; and Medical Laboratories, Collis P. Huntington Memorial Hospital, Massachusetts General Hospital, Boston, Mass.

Using the Hardy reflectance spectrophotometer, an attempt has been made to study quantitatively the changes in pigmentation which occur following ACTH and cortisone administration. A record of the changes in skin color, throughout the visible spectrum has been made in 7 patients with Addison's disease prior to, and following cortisone ther-

apy; in 7 patients before and after complete adrenalectomy, in 5 patients without adrenal disease receiving ACTH; and in 3 patients receiving large doses of ACTH and cortisone in successive courses. Using known color characteristics of skin pigments, information concerning changes in melanin or melanoid concentrations, hemoglobin content and hemoglobin oxygenation may be obtained. The characteristics of desoxycorticosterone-treated Addisonians prior to cortisone therapy are increased melanin concentration, decreased hemoglobin content and decreased hemoglobin oxygenation; with cortisone, all these revert toward normal. In patients without adrenal disease, treatment with ACTH produces an increased melanin content which can be differentiated from the lightening associated with fluid retention. No melanin increase is seen, however, following cortisone therapy.

Bioassay in frogs demonstrates a high intermedin content of the ACTH preparations used. Evidence suggesting the presence of excess circulating intermedin in Addison's disease will be discussed.

23. Hormones and Brain Excitability.

Dixon M. Woodbury.

From the Department of Pharmacology, University of Utah College of Medicine, Salt Lake City, Utah.

The adrenocortical steroids influence the electroshock seizure threshold (EST) in rats as follows: desoxycorticosterone acetate (six 15-mg. pellets), marked increase; 11-desoxy-17-hydroxycorticosterone (2 mg./day), slight increase; corticosterone acetate (2 mg./day), no change; 11-dehydrocorticosterone acetate (2 mg./day), slight decrease; 17-hydroxycorticosterone acetate (2 mg./day), moderate decrease; and cortisone acetate (2 mg./day), marked decrease. ACTH (0.5 U.S.P. unit/day) produces a slight increase in EST. Adrenalectomy is followed by a decrease in EST; this decrease is accentuated by cortisone acetate in a dose which prolongs the life of the animal. Thyroidectomy and propylthiouracil (2 per cent in food) in rats increase EST and decrease susceptibility to metrazol-induced seizures. Conversely, thyroxine (100 micrograms/day) decreases EST and increases susceptibility to metrazol-induced seizures. Hypophysectomy produces a slight elevation (5 per cent) during the first few postoperative days; however, the threshold decreases progressively thereafter, and at twenty-six days is at the preoperative level. The data clearly demonstrate that the secretions of the adrenal cortex and the thyroid markedly influence the activity of the central nervous system. Naturally occurring and induced endocrinopathies in man are associated with alterations in activity of the central nervous system which conform with the above observations in rats.

24. A Comparison of Effects of Single Injections of ACTH and Epinephrine Upon Adrenal Cortical Activity.

William McK. Jeffries, Alfred K. Bochner (by invitation) and *Ralph I. Dorfman.*

From the School of Medicine, Western Reserve University, Cleveland, Ohio.

The effect of single injections of 25 mg. of ACTH i.m. and 0.3 mg. of epinephrine s.c. upon urinary excretion of Na, K and 17-ketosteroids, as well as upon circulating eosinophils, was studied, utilizing the time relationships of the Thorn test, in 100 normal young men. Consistent increases in K and 17-ketosteroid excretion occurred with the ACTH test. With epinephrine, however, only one third showed an increase in excretion of these substances, and in these, the magnitude of the change was of questionable significance; yet the circulating eosinophils decreased in the expected fashion. Three cases of Addison's

disease had no increase in K or 17-ketosteroid excretion following ACTH, and no consistent changes occurred with epinephrine. Following a single alarming stimulus, the change in excretion of these substances was found to be similar to that following ACTH. From these studies it is evident that the changes in urinary excretion of Na, K and 17-ketosteroids in the Thorn test give a more complete picture of the pattern of adrenal response, and that the customary dose of epinephrine used in the screening for pituitary-adrenal function does not elicit a pattern of response similar to that produced by 25 mg. of ACTH.

25. The Effects of Desoxycorticosterone Acetate and Saline on the Bilaterally Adrenalectomized Rhesus Monkey.

Frederick G. Hofmann (by invitation), *Ernest Knobil* (by invitation), *Thomas L. Jones* (by invitation) and *Roy O. Greep.*

From Harvard University, Boston, Mass.

The physiologic effects of these agents have been evaluated in monkeys exhibiting before treatment severe adrenal insufficiency. Blood levels of sodium, potassium, chloride, nonprotein nitrogen, amino-acid nitrogen, glucose, and inorganic phosphorus were measured at 3-day intervals, as were hematocrit and body weight. General appearance and appetite were also noted. Cortisone (2 mg./Kg./day for six to twelve days) generally restored the blood constituents to normal, except in the case of NPN and electrolytes, in which instances the response varied. Food intake was not improved and the animals continued to lose weight. Desoxycorticosterone acetate (2 mg./Kg./day for nine to twenty-seven days) produced an increase in appetite and either no further loss, or some gain in body weight. Blood glucose levels remained generally unchanged, the sodium rose to abnormal concentrations and there was marked hemodilution. All other measured blood constituents were returned to normal. Monkeys given 0.9 per cent NaCl as drinking fluid displayed a normal blood picture, except for depressed glucose levels. Appetite improved and body weight loss was checked. Enhanced fluid intake and localized edema were noted during the first week of the therapy period.

26. Effect of Cortisone on the Abnormal Intravascular Distribution of Water in Addison's Disease.

Joseph F. Dingman (by invitation), *David H. P. Streeten* (by invitation) and *George W. Thorn.*

From the Department of Medicine, Harvard Medical School and the Medical Clinic, Peter Bent Brigham Hospital, Boston, Mass.

The intravascular distribution of water load in normal subjects, Addisonian, and completely adrenalectomized patients was determined by hourly estimations of mean corpuscular hemoglobin concentration (MCHC). These changes provide an index of variations in erythrocyte size, assuming that the red cell mass remains constant. In normal subjects following a water load, the MCHC fell 2.00 per cent during the first hour, indicating corpuscular swelling, with a return to normal by the second hour. In Addisonians on desoxycorticosterone treatment only, erythrocyte swelling was greater and more prolonged and far exceeded the experimental error. MCHC depression averaged 3.46 per cent in one hour and 2.97 per cent after four hours. Oral cortisone, administered before the water load, reduced the degree and duration of erythrocyte swelling progressively as the dose was increased from 25 to 100 and 200 mg. These cellular changes often occurred without improvement in water diuresis, indicating that cortisone protects the cells

from excessive hydration independently of its effects on urine output. A patient with Cushing's syndrome was studied in whom the erythrocyte volume actually decreased during a water test, despite a subnormal diuretic response.

27. A Quantitative Study of the Prevention of ACTH-Induced Sodium Retention by Potassium Salts.

Grant W. Liddle (by invitation), *Peter H. Forsham* and *Leslie L. Bennett.*

From the University of California School of Medicine, San Francisco, California.

The effect of potassium salts upon ACTH-induced sodium retention has been studied in 9 human subjects on constant intake. The sodium retention which usually occurs during treatment with ACTH may be completely prevented, or once induced, reversed by the concurrent administration of potassium amounting to 150 and 350 mEq. per day. When sodium diuresis occurs it far exceeds the magnitude of the simultaneous potassium retention. Potassium-induced sodium diuresis does not depend upon previous retention of sodium, since the percentage rise in sodium excretion is identical on high and low sodium intakes. These effects occur with either potassium chloride or potassium acetate. Potassium appears to effect sodium diuresis by its action on the kidney rather than on the adrenal cortex; for it also prevents cortisone-induced sodium retention and it was shown that potassium itself fails to modify other indices of adrenocortical activation by ACTH.

FRIDAY, JUNE 6, 1952

9:00 A.M.: CALCIUM METABOLISM; SUPPORTING TISSUES

CHAIRMEN: JOHN E. HOWARD AND EPHRAIM SHORR

28. Alterations in Calcium Metabolism in Patients with Osteolytic Tumors.

O. H. Pearson, C. D. West (by invitation), *V. P. Hollander* (by invitation) and *G. C. Escher.*

From the Sloan-Kettering Institute, New York, N. Y.

Growth of osteolytic tumors is, by definition, associated with destruction of normal bone. Thus calcium balance studies should provide, as a first approximation, a quantitative measure of the rate of osteolytic tumor growth.

Studies in 6 patients with metastatic osteolytic breast carcinoma have revealed negative calcium balances of 0.2 to 1.6 grams daily. Hypercalcemia existed in all patients whose daily calcium loss was 0.5 gram or more. Oophorectomy in one patient, and adrenalectomy in another, was followed by prompt cessation of calcium loss, subsequent calcium retention, and objective clinical remission. Estradiol benzoate administered to another patient resulted in a prompt increase in calcium loss with the development of hypercalcemia. Cessation of the steroid was followed by prompt disappearance of hypercalcemia and a sharp decline in calcium loss. These observations indicate stimulation of the osteolytic process by estrogen. Cyclic spontaneous hypercalcemia and hypercalcuria, apparently related to the menses, has been observed in another patient, suggesting that endogenous alteration in endocrine balance altered the rate of osteolytic tumor growth.

These observations indicate that calcium excretion data provide an objective index of the rate of growth of osteolytic tumors and permit evaluation of therapeutic measures before the clinical response may be manifested.

29. Studies Regarding the Mechanism of Bone Disease in the Detoni-Fanconi Syndrome.

L. H. Kyle, W. H. Meroney, III (by invitation) and *M. E. Freeman* (by invitation).

From the Department of Medicine, Georgetown University School of Medicine; the Medical Service, Walter Reed Army Hospital and the Army Medical Service Graduate School, Washington, D.C.

The cause of the bone disease which occurs in association with renal glycosuria, aminoaciduria and hyperphosphaturia has never been clearly identified. Possible mechanisms include: 1) loss of specific amino acids necessary for normal bone metabolism, 2) calcium deficiency resulting from inability of the overloaded renal base-sparing mechanisms to operate efficiently, and 3) phosphate deficiency secondary to decreased tubular reabsorption of that substance. A 42-year-old man with severe osteomalacia and other defects characteristic of the Fanconi syndrome has been studied in an attempt to explain the pathogenesis of the bone disease. It was shown that neither the glycosuria nor aminoaciduria were of sufficient degree to effect negative nitrogen balance, or suggest undue loss of any specific amino acid. The addition of an acid load resulted in a comparable increase of urinary ammonia. While he was on a neutral ash diet the patient's urinary ammonia production and titratable acidity were increased and he was in negative calcium and phosphorus balance. The addition of base markedly decreased the urinary titratable acidity but did not decrease the calcium or phosphorus excretion. Administration of vitamin D resulted in a positive calcium balance but the phosphorus loss became more marked. When extra phosphate was added to the diet, phosphorus balance became positive, calcium excretion diminished and there was a moderate elevation of the serum phosphorus level.

These observations indicate that the disease associated with glycosuria and aminoaciduria can best be explained on the basis of urinary loss of phosphorus. The previously observed lack of response to vitamin D in these patients can be attributed to the fact that, although temporary calcium retention follows application of vitamin D therapy, phosphorus excretion becomes more pronounced.

30. The Use of Intravenous Calcium as a Measure of Activity of the Parathyroid Glands.

John Eager Howard, Theda R. Hopkins (by invitation) and *Thomas B. Connor* (by invitation).

From the Department of Medicine, The Johns Hopkins University, Baltimore, Maryland.

Intravenous calcium gluconate, in a dosage of 8 to 30 mg. of calcium per kilogram, was given over a 4-hour period to a group of normal persons, to patients with hyperparathyroidism due to parathyroid adenomata, to patients with hypoparathyroidism following thyroidectomy, and to patients with various types of bone disease. Serum calcium and inorganic phosphorus levels were followed. Urine calcium and phosphorus determinations were made for a 24-hour control period and for the test day. The normal group revealed a characteristic response to intravenous calcium, *viz.*, a rise in serum phosphorus and a fall in urine phosphorus. The hyperparathyroid group showed little change in serum phosphorus and either little change or a rise in urine phosphorus with reversion to the normal pattern after parathyroid tumor removal. The hypoparathyroid group showed little change in serum phosphorus and a marked rise in urine phosphorus. It is postulated that elevation of the serum calcium level temporarily depresses parathyroid activity in normal persons as manifested by a rise in serum phosphorus and a fall in urine phosphorus, and that this response is not obtained in patients with parathyroid

adenomata since tumors are not under normal homeostatic control, nor in patients with hypoparathyroidism since little if any parathyroid tissue is functioning.

31. Calcium Loss in Paralytic Poliomyelitis and its Quantitative Relationship to the Development of Demonstrable Osteoporosis.

G. Donald Whedon and *Ephraim Shorr.*

From the Russell Sage Institute of Pathology, the Department of Medicine, Cornell University Medical College, and The New York Hospital, New York, N. Y.

Metabolic balances were carried out for six to ten months in 9 adult patients with acute anterior poliomyelitis. Paralysis was generally marked and extensive. Studies on 4 patients were begun early in the acute phase. As soon as possible constant diets were instituted, calcium intake being 920 mg. daily.

A progressive increase in both urinary and fecal calcium was noted. Maximum urinary calcium for a 6-day metabolic period ranged from 391 to 789 mg./day (the mean being 572 mg./day) and occurred, on the average, five weeks after onset. Urinary calcium remained above 250 mg./day for five and a half months. The mean maximal negative calcium balance (−0.540 gm./day) occurred during the ninth week. Total calcium losses ranged from 17.0 to 113.4 Gm., the mean being 58.0 Gm. The duration of negative calcium balance was seven months, the patients having been recumbent for six months on the average.

Initial x-ray evidence of osteoporosis, usually patchy demineralization and coarsening of the trabeculae in the lower extremities regional to points, appeared approximately three months after onset, with an average loss of 2 per cent of estimated total body calcium. Total calcium losses for seven months of negative balance averaged 4 per cent and ranged up to 8.5 per cent without detectable changes in the spine.

32. Strontium as an Adjuvant to Calcium in the Remineralization of the Skeleton in Man.

Anne C. Carter and *Ephraim Shorr.*

From the Russell Sage Institute of Pathology, the Department of Medicine, Cornell University Medical College and The New York Hospital, New York, N.Y.

The value of strontium as an adjuvant to calcium in the remineralization of the skeleton has been established by metabolic studies in 10 patients with menopausal and other types of osteoporosis. Strontium was given as the lactate, usually in amounts equivalent to 1.7 Gm. of Sr daily, to some patients for as long as five years. No toxic effects were encountered. When administered along with an equal amount of Ca, the retention of Sr was usually as great as, and frequently greater than, that of Ca, with no adverse effect on Ca storage. When maximal Ca storage had been achieved by raising Ca intake, addition of Sr resulted in retention of the latter, usually with no alterations in nitrogen balance. Calculations from balance data indicated storage of Sr largely as tristrontium phosphate. Strontium storage was augmented in similar fashion by vitamin D, estrogens and androgens. Symptomatic relief and return to activity occurred at a rate corresponding to the magnitude of combined Ca and Sr storage. Preliminary results indicate that the rate of Sr loss after discontinuing its administration can serve as a measure of lime salt turnover in bone, without the hazards associated with the use of radioisotopes.

33. Refractoriness to Antitetanic Therapy in a Case of Surgical Hypoparathyroidism.

Raymond W. Blohm, Jr. (by invitation), *Otto A. Wurl* (by invitation), *James O. Gillespie* (by invitation) and *Roberto F. Escamilla.*

From the Letterman Army Hospital, San Francisco, California.

A 30-year-old housewife was hospitalized because of symptoms of hyperthyroidism and study showed typical exophthalmic goiter. After preparation with propylthiouracil and Lugol's solution, subtotal thyroidectomy was performed without incident. Thirty-six hours later, carpopedal spasm occurred. One parathyroid gland was found in the excised tissue. Treatment with diet, dihydrotachysterol, vitamin D, calcium and amphojel seemed effective for two weeks; but then hypoparathyroidism progressed in spite of increasing doses of the medications, with the daily dose of dihydrotachysterol reaching a level of 18 cc. Progressive exophthalmos also developed, with mild hypothyroidism and temporary paralysis of the left vocal cord. A latent tetany persisted and occasional severe tetany required control by intravenous calcium. Metabolic studies over a period of four months showed a consistently positive calcium balance in spite of treatment with parathormone and dihydrotachysterol. The patient has now been followed for two years and continues in latent tetany, with occasional severe tetany, in spite of a daily routine which includes special diet, oral calcium, amphojel, thyroid and 1,000,000 units of vitamin D. She has become pregnant and is approaching term. Data will be given concerning the patient and the child after delivery.

34. Effect of TSH and ACTH on Amino-Acid Metabolism.

D. M. Bergenstal and *K. Lugibihl* (introduced by A. T. Kenyon).

From the Department of Medicine, University of Chicago, Chicago, Illinois.

A catabolic state of protein metabolism induced by thyrotropin and adrenocorticotropin in human subjects in metabolic balance studies is associated with a sharp rise (100 to 300 per cent) in urinary free amino acid nitrogen excretion. The excretion curve of amino nitrogen returns to baseline levels within forty-eight hours after ACTH is stopped, whereas that of total urinary nitrogen may continue elevated for six to nine days. Thyrotropin gives a different response, in that the total nitrogen and the amino nitrogen excretion curves are closely parallel. During the stress involved by thyrotropin administration there was no evidence of increased adrenal activity. A patient with treated myxedema given ACTH showed a normal response in amino acid nitrogen excretion. No increased participation of the thyroid hormone was necessary for the response of ACTH. If the proteins of the diet are increased or decreased, the calories remaining the same, the excretion of amino acid nitrogen and total nitrogen during ACTH administration is unaltered. There may be at least two mechanisms contributing to the observed effects of ACTH: 1) Increased catabolic effect on protein metabolism, and 2) alterations in renal tubular capacity for handling amino acids.

35. Dietary Modification of the Effects of ACTH and Cortisone.

John W. Partridge (by invitation), *Lenore Boling* (by invitation), *Florence Olson* (by invitation), *Eleanor Kipp* (by invitation) and *Laurance W. Kinsell.*

From the Institute for Metabolic Research of the Highland Alameda County Hospital, Oakland, California.

It is well known that the limitation of sodium intake in patients receiving ACTH and cortisone will result in significant decrease of fluid retention. Despite such sodium limita-

tion, however, many undesirable effects of hormonal administration are noted in many patients.

In the course of studies dealing with certain aspects of fat metabolism, it was found that:

1. Patients with rheumatoid arthritis, receiving a very high fat, low carbohydrate diet, had significant improvement in the symptoms of their rheumatoid arthritis; and that symptoms tended to return when the carbohydrate content of the diet was increased.

2. The use of such a high fat, low carbohydrate diet (suitably supplemented with minerals and vitamins) appeared to result in a relatively greater therapeutic effectiveness of administered ACTH and cortisone, and a relatively lesser development of untoward manifestations.

3. In some patients significant differences in 17-ketosteroid excretion occurred with a given dose of ACTH when the composition of the diet was changed.

The present report summarizes the observations thus far obtained, and considers the possible implications of these observations, with particular reference to a possible change in the metabolism of adrenal steroids in relation to dietary composition.

36. Hormonal Interaction Regulating Granulation Tissue Development.

Matthew Taubenhaus, Bernard Taylor (by invitation) and *John V. Morton* (by invitation).

From the Departments of Metabolic and Endocrine Research and Pathology, Michael Reese Hospital, Chicago, Illinois.

In previous experiments, it was shown that cortisone inhibits locally the granulation tissue accumulating around turpentine abscesses. Estradiol, which had been shown to inhibit granulation tissue systematically, when mixed with cortisone and turpentine, does not show an additive inhibitory effect. Methylandrostenediol behaves similarly to estradiol, but its effect is much less pronounced. It resembles testosterone in its action upon granulation tissue. Desoxycorticosterone stimulated granulation tissue formation in previous experiments only if the animals were pretreated with this substance. The appearance of this tissue, however, differed from the granulation tissue, in that the fibroblasts assumed a stellate appearance, the ground substances became hyalinized and fewer fibers were observed. In recent experiments, animals were pretreated with desoxycorticosterone for two weeks, then turpentine mixed with cortisone was injected on the right side and turpentine alone placed on the left side. The ordinarily observed inhibition by cortisone was counteracted by desoxycorticosterone and ample granulation tissue was observed on both sides. The appearance of this tissue, however, differed from the regular one as described before. Anterior pituitary growth hormone administered to animals which had been injected with turpentine alone on the left side, and turpentine and cortisone on the right, counteracted the cortisone inhibition nearly completely and produced normal looking granulation tissue. It is concluded that growth hormone is a true antagonist of cortisone, and that the inhibition by the latter may be due to local interference with the stimulating effect of growth hormone.

37. The Effects of ACTH and Cortisone on the Development of Experimental Exophthalmos in the Guinea Pig.

A. W. Ludwig (by invitation), *N. F. Boas* (by invitation) and *L. J. Soffer.*

From the Mount Sinai Hospital, New York, N. Y.

Acute experimental exophthalmos was induced in intact and in thyroidectomized

guinea pigs by the administration of 2 mg./day of purified adenohypophyseal thyrotropic factor. Histologic and histochemical study of the orbital tissues of these animals revealed that the development of exophthalmos is associated with the deposition of large quantities of mucinous ground substance in the retrobulbar connective tissues, in the form of amorphous metachromatically staining extracellular material. These findings were confirmed by the demonstration of significant increases in the hexosamine and water contents of these tissues with the appearance of exophthalmos. The total water content of normal orbital tissues was 80.6 per cent ± 0.4; and the hexosamine content, 0.70 ± 0.03 mg./100 mg. dry weight. In exophthalmic animals these rose to 86.9 per cent ± 0.5; and 1.22 ± 0.11 mg./100 mg., respectively.

Treatment of the experimental animals with varying doses of ACTH and cortisone (2 to 5 mg./day) failed to inhibit the development of gross exophthalmos, or to produce any notable changes in the histologic picture. A slight diminution of the increases in hexosamine and water content was observed in exophthalmic guinea pigs to which ACTH and cortisone had been administered.

38. Serum Mucoprotein and Mucoproteins of Connective Tissue of the Skin in Generalized Myxedema.

R. E. Mancini (by invitation), *J. C. Garberi* (by invitation) and *F. A. de la Balze.*
Tercera Catedra de Clinica Medica, Facultad de Medicina, and Instituto Nacional de Endocrinologia, Buenos Aires, Argentina.

Morphologic and histochemical methods were applied to biopsy of the skin of the arm in 9 cases of myxedema of thyroid origin; results were compared with those obtained in the skin of 5 normal subjects. The following techniques were used: Hotchkiss-McManus, toluidine blue, colloidal iron, orcein, silver impregnation and Masson's trichromic. The level of a serum mucoprotein fraction detected by Winzler's method was assayed in all patients and also in 22 normal subjects as a control. In the 5 cases of severe myxedema we observed: a) partial atrophy of the basal membrane into its two components, reticular fibers and homogenous substance; b) in the papillar corium, partial disappearance of the reticular and elastic fibers, but an increase in the amorphous substance (partially digested by testicular hyaluronidase) and abundant juvenile fibroblasts; c) swelling of the inner corium and gradual disintegration into its collagenous and elastic fibers with a great increase in the amorphous substance (completely digested by hyaluronidase); and d) a significant increase in serum mucoprotein. In the 4 cases of moderate myxedema we observed: a) intense swelling of the collagenous fibers and bundles of the inner corium and a small increase of amorphous substances (completely digested by hyaluronidase); and b) an unmodified level of serum mucoprotein.

39. The Adrenal Cortex and Sulphur Metabolism

Joseph W. Goldzieher, William B. Rawls (by invitation) and *Max A. Goldzieher.*
From the Research Department, St. Clare's Hospital, New York, N. Y.

Older data suggested a thiopexic function of the adrenals. This activity has been investigated, using a modified amperometric titration for sulfhydryl measurement. Various sulfur-bearing compounds (*l*-cystine, *l*-cysteine, glutathione) and analogous non-sulfur amino acids (serine, threonine) were administered to rats and the SH content of adrenals, liver and diaphragm studied. Substantial SH pickup was observed in the adrenal. Intravenous cysteine did not deplete adrenal ascorbic acid in the hypophysectomized rat.

L-cystine was fed to 68 patients for periods of from four to eight weeks; significant

depression of 17-ketosteroid and neutral reducing corticoid excretion was observed. This was not affected by pretreatment or cotreatment with ascorbic acid.

ACTH was administered to rats in various dosage schedules and significant depletion of adrenal SH was demonstrated. Intravenous infusions of ACTH were given to 15 patients for one to three days and changes in blood SH and ascorbic acid studied. Significant depression of blood SH occurred at four hours, with a return to normal at eight hours despite continued infusion. Correlated changes of blood ascorbic acid could not be demonstrated.

Liver glycogen studies on sulfur-treated animals are in progress.

40. Nucleic Acid Changes in Tissues of Normal and Diphtheria Intoxicated Guinea Pigs with Cortisone.

Leon T. Atlas and *Kurt Benirshke* (introduced by George W. Thorn).

From the Department of Medicine and Pathology, Peter Bent Brigham Hospital, Boston, Mass.

Following four to nine days of cortisone therapy, 104 guinea pigs were inoculated with diphtheria toxin; 103 animals received toxin but no cortisone. Fifty-four cortisone-treated and 50 untreated controls received heat-inactivated toxin. Cortisone, 5 mg. intramuscularly twice daily, was continued for fifteen days. Animals were autopsied immediately after death or sacrificed after fifteen days and their tissues prepared for pathologic examination and estimation of nucleic acids.

For the first five days following toxin administration, there was a significant increase in duration of survival among the cortisone-treated group. Thereafter, deaths in these cortisone-treated animals increased, so that the mortality in both treated and untreated groups was similar by the end of fifteen days. Although there was no change in ribonucleic acid content per unit dry weight of liver, in both inactivated toxin control and toxin surviving animals, cortisone lowered the desoxyribonucleic acid content. Death from toxin increased the liver desoxyribonucleic acids. This delay in lethal effect from diphtheria toxin and the decrease in desoxyribonucleic acids of tissue produced by cortisone suggests that the diverse manifestations of cortisone may result from a single mechanism in the synthesis, use or breakdown of desoxyribonucleic acids.

2:00 P.M.: PRESIDENTIAL ADDRESS.

Some Basic Hormone Problems.

Gregory Pincus

2:30 P.M.: STEROIDS; ASSAY, METABOLISM AND PHYSIOLOGY

CHAIRMEN: ROY HERTZ AND WILLIAM T. SALTER

41. The Effects of Graded Doses of Cortisone on Circulating Eosinophils in the Intact Mouse.

F. Halberg and *M. B. Visscher.*

From the Department of Physiology, University of Minnesota, Minneapolis, Minn.

A quantitative assay of 11,17-oxycorticoids is based on tests made during the ascending phase of the diurnal eosinophil rhythm. The index employed is the percentage change in the numbers of eosinophils in intact ZBC male mice over a 4-hour period. The mice are injected at 02:00 after obtaining tail blood for counting.

Handling for two bleedings does not prevent the rise in numbers of eosinophils from 02:00 to 06:00. Injection of 0.1 cc. of saline, subcutaneously, may prevent the rise, but does not induce an average fall in numbers of eosinophils greater than 50 per cent. In 10 independent studies, cortisone in 5γ doses induced a fall significantly greater than the fall following saline. The increment in response from saline to 5γ of cortisone was 49 ±18 per cent ($P_T < .01$—) and from 5γ to 10γ it was 35±10 per cent ($P_T < .01$). Comparison of 6-, 8-, and 20-week-old mice suggests that 8-week-old animals are satisfactory for assay use.

42. Bioassay of Adrenal Steroids, Based on Eosinophil Counts in Adrenalectomized Mice. A Statistical Study.

Eugenia Rosemberg (by invitation), *Jerome Cornfield* (by invitation) and *Evelyn Anderson.*

From the National Institutes of Health, Bethesda, Maryland.

Several investigators have used a method of bioassay of adrenal steroids based on the fact that adrenalectomized mice subjected to graded doses of cortisone show a graded decrease in eosinophil count. Our findings have indicated that an increase in the eosinophil count is characteristic over the period from 11 A.M. to 3 P.M. when less than 1.0 mcg. of cortisone acetate is given. With saline alone there is a median increase of 70 per cent; with epinephrine (5–20 mcg.), 200 per cent. The assay method to be described is based on a measure of change in the eosinophil count which includes not only the decreases but also the increases. The test animals are pretreated with 5 mcg. of epinephrine. The ratio of eosinophil count four hours after the administration of cortisone acetate to that before administration is computed for each animal and the median ratio computed for each dose level. These median ratios are linear against log dose over the entire range of doses tested (0.247–20.0 mcg. of cortisone acetate) and provide the basis for an assay. If one computes the intrinsic error of the assay as the ratio of the standard deviation to the change in response per tenfold dilution, usually denoted by *lambda*, one obtains a value of 0.6 in the range of doses, 1.0 to 10.0 mcg. These results apply when animals are pretreated with 5 mcg. of epinephrine. When animals are not pretreated with epinephrine, the value of *lambda* is almost twice as great as when they are pretreated. When no epinephrine is given, the number of animals required to reach a given level of precision is consequently three to four times as great as when epinephrine is given. Urine from patients is being assayed by this method.

43. Assay of ACTH Activity Based on Eosinopenic Response of Normal Mice.

Robert S. Speirs (introduced by R. K. Meyer).

From the R. B. Jackson Memorial Laboratory, Bar Harbor, Maine.

Intact BBF_4 mice were injected intraperitoneally with ACTH suspended in water, gelatine and sesame oil and eosinophil counts were made prior to and at 6, 10, 18, 24, 48, 72 and 96 hours after the injection. In all groups, including the controls, there was a marked eosinopenia 6 hours following the injection of 2 I.U. of ACTH. Recovery occurred by 10 to 18 hours in the group receiving the ACTH suspended in 0.25 ml. of saline, in 18 to 24 hours in the group receiving the ACTH suspended in 0.1 ml. of gelatine, and in 48 hours in the group receiving the ACTH suspended in 0.05 ml. of sesame oil. In another group which received 4 mg. of ACTH suspended in sesame oil, there was a complete absence of eosinophils at 24 hours and a 98 per cent eosinopenia at 48 hours. Recovery did not occur until 72 hours after the injection. All the animals that received control

injections showed a complete recovery by 24 hours. These data suggested that an assay could be developed for gelatine and oil suspensions of ACTH in which the 24-hour response of the circulating eosinophils was used. This type of assay has the advantage of indicating the amount of 11-oxycorticosteroid hormone released and the period of time during which it is released from the adrenal cortex.

44. The Assay of 11-Oxycorticosteroids by Means of Liver Reducing Substances in Adrenalectomized Animals.

N. R. Stephenson, and *J. C. Taylor* (introduced by L. I. Pugsley).

From the Food and Drug Laboratories, Department of National Health and Welfare, Ottawa, Canada.

A study has been made of factors influencing the precision of the bioassay of 11-oxycorticosteroids, such as the measurement of the response, route of administration and diet. It has been shown that the amount of total reducing substances in the liver of adrenalectomized animals serves as a satisfactory criterion for estimating the response to graded doses of either cortisone acetate, hydrocortisone acetate or adrenal cortical extracts. The slopes of the log dose-response lines for both liver reducing substances and liver glycogen have been found to be not significantly different, indicating that both criteria measure the same response. Total reducing substances in the liver may be determined more rapidly and with less experimental variation than liver glycogen. The livers of the experimental animals are hydrolysed in IN sulfuric acid for thirty minutes, broken up with a glass rod and autoclaved at 15 pounds pressure for fifteen minutes. The resulting digest for each liver is neutralized, brought to a specified volume, and a suitable aliquot is deproteinized with zinc hydroxide (Somogyi). Total reducing substances in the filtrate may be estimated either by means of copper reduction (Nelson-Somogyi procedure), or by the Anthrone method. The effects of the route of administration and of diet on the precision and sensitivity of the assay are discussed.

45. Erucic Acid as the Factor in Rape Oil Affecting Rat Adrenal Cholesterol.

K. K. Carroll, and *R. L. Noble* (introduced by J. B. Collip).

From the University of Western Ontario, London, Ontario, Canada.

Feeding rape oil to rats as 25 per cent by weight of the diet increases the cholesterol content of their adrenals to three to five times that of normal rats (Carroll: *Endocrinology* **48:** 101, 1951). This effect has been shown to be produced mainly by erucic acid, one of the component fatty acids of rape oil, and a similar response may be obtained by feeding this acid as 15 per cent of the diet for three weeks. Other naturally occurring fatty acids fed at the same or higher levels have given increases in adrenal cholesterol of smaller magnitude. Various tests of adrenal function have been applied to rats fed on rape oil and no evidence of abnormality resulting from the accumulation of cholesterol in the adrenals was obtained. Species variations have been observed, in that feeding rape oil produced little or no change in the level of adrenal cholesterol in guinea pigs, rabbits and chickens, whereas increases were obtained in rats and mice.

46. Utilization of C^{14}-Acetate for Cholesterol and Steroid Hormone Synthesis in the Human.

Leon Hellman, Robert R. Rosenfeld, David K. Fukushima, T. F. Gallagher and *Konrad Dobriner.*

From the Sloan-Kettering Institute for Cancer Research, New York, N. Y.

Serial measurements were made of the incorporation and disappearance of radio-

activity in the serum cholesterol, the fecal cholesterol, and the urinary androsterone, etiocholanolone and dehydroisoandrosterone, following the administration of radioactive acetate to a man with a massive adrenal tumor and extensive hepatic metastases. The rates of transformation were consistent with the interpretation that the urinary steroids were derived from serum cholesterol and that this was in turn in equilibrium with the total body pool of cholesterol, including the fecal cholesterol. When the study was terminated by the death of the subject (thirty days after initiation), the cholesterol of liver, adrenal blood and muscle had the same specific activity. Initially, the urinary dehydroisoandrosterone had a slightly higher specific activity than serum cholesterol and might thus arise in part directly from administered acetate. Further details of these studies are described.

47. Biogenesis of Androgens in the Human Testis.

K. Savard (by invitation), *R. I. Dorfman* and *E. Poutasse* (by invitation).

From the Worcester Foundation for Experimental Biology, Shrewsbury, Mass., and the Cleveland Clinic Foundation, Cleveland, Ohio.

The biosynthesis of androgens has been studied by the perfusion of the human testis with pituitary gonadotropin and C^{14}-carboxyl-labeled acetate. Human testes obtained from patients with prostatic carcinoma were cannulated prior to extirpation, flushed with citrated physiologic saline on removal, and maintained at from 2° to 5° C. until perfusion. The good condition of the glands during the perfusion is evidenced by the fact that they responded to gonadotropin during perfusion and the subsequent histologic examination. Blood containing 2.9×10^7 counts of C^{14}-carboxyl-labeled acetate per 100 ml. was perfused through the human testis at the rate of 3 ml. per minute, with and without gonadotropin. A crude index of gonadotropin activity is shown in the table by the increased radioactivity following the addition of gonadotropin.

	Material extracted from testis perfusate, counts/min./100 ml. of blood	
	Total solids	Neutral steroid fraction
Perfused blood	28,000	4,300
Perfused blood containing gonadotropin	124,000	9,550

The neutral steroid fraction prepared from the perfusates contained radioactivity ranging from 500 to 2,400 counts/mg./min. This fraction, to which carrier was added, was fractionated by means of silica-gel chromatography, digitonin, Girard's ketone reagent, and paper chromatography. Radioactivity was determined at each stage. When the recovered steroid was radioactive, recrystallization combined with paper chromatography of both free steroids and their derivatives, was the method employed for further assurance of purity. By these means the conversion of acetate to testosterone and delta-4-androstene-3,17-dione by the human testis has been demonstrated. No evidence could be found for the presence of delta-5-pregnene-3-beta-ol-20-one or androstane-3-17,dione in the perfusates.

48. Corticosteroid Metabolism in Isolated Perfused Rat Livers.

O. Hechter, M. M. Solomon (by invitation), *I. A. Macchi* (by invitation), *E. Caspi* (by invitation) and *M. Feinstein* (by invitation).

From the Worcester Foundation for Experimental Biology, Shrewsbury, Mass.

To evaluate the enzymatic capacity of hepatic tissue to transform corticosteroid hor-

mones, a variety of corticosteroids have been perfused through rat livers with citrated bovine blood. Using 10 mg./100 ml. of blood, cortisone, 11-desoxycorticosterone, 17-hydroxy-11-desoxycorticosterone and 17-hydroxy-corticosterone were recycled through the organ, so that the effects of cycling 1, 5, and 10 times were studied. As a control, the effect of blood incubation at 37° upon the corticosteroids was determined. Despite minor quantitative differences, the qualitative results obtained with all of these steroids were similar and may be summarized as follows:

a) The bulk of the corticosteroid incubated in *blood*, without liver perfusion, is recovered unchanged, but in all cases there is evidence by paper chromatography, of the appearance of new compounds in minor amount.

b) The added steroid (measured after paper chromatography) disappears rapidly after liver perfusion so that after one circulation about 50 per cent is converted, and more than 90 per cent is transformed in 5 cycles.

c) Approximately 40 to 50 per cent of the transformation products formed in 5 to 10 circulations retain the ketol side-chain, but no longer possess the α-β unsaturated ketone grouping in ring A. These represent, in the main, a multiplicity of substances of greater polarity than the original steroid. The remaining transformation products, known to be present on the basis of radioactive studies, are being investigated.

These findings of the rapid hepatic transformation of corticosteroids, including cortisone, taken together with the clinical finding that cortisone is approximately as active orally as by parenteral injection, suggest that metabolites of cortisone, formed in liver, retain biologic activity.

49. Levels of 17-Hydroxycorticosteroids Following Intravenous Infusion of Epinephrine Into Normal Men.

Don H. Nelson, Avery A. Sandberg, J. G. Palmer and *E. Myles Glenn* (introduced by Leo T. Samuels).

From the Departments of Biochemistry and Medicine, University of Utah College of Medicine, Salt City, Utah.

Epinephrine in quantities ranging from 1 to 3 mg. was administered intravenously to normal young men over a period of four hours. Although the marked fall in eosinophils and lymphocytes with increase in neutrophils described by Recant *et al.* (*Fed. Proc.* **7**: 99, 1948) occurred, there was no measurable rise in blood levels of 17-hydroxycorticosteroids during or immediately following the infusion. The effect on blood elements was equal to that seen following 200 mg. of compound F acetate given orally, when blood levels of 17-hydroxycorticosteroids rise from a top normal level of 20γ per 100 ml. of plasma to 80–120γ per cent.

50. The Effect of Cortisone on the Excretion of 17-Ketosteroids in Patients with Adrenal Tumor.

Eleanor H. Venning, C. J. Pattee and *Frances McCall* (by invitation).

From the McGill University Clinic, and the Children's Memorial Hospital, Montreal, Canada.

The effect of cortisone on the excretion of 17-ketosteroids was studied in 3 cases of adrenogenital syndrome, 2 children and 1 adult, prior to the removal of an adrenal tumor. A fourth patient showing clinical features of both the adrenogenital and Cushing's syndromes also received cortisone. At operation this patient was shown to have an unilateral hyperplasia of the adrenal gland. Cortisone in doses ranging from 100 to 200 mg.

caused a slight temporary reduction in the excretion of 17-ketosteroids in 3 of the cases, but this effect was not maintained and on continued administration of cortisone the 17-ketosteroid excretion rose again. In none of the cases could the level of 17-ketosteroid excretion be lowered to within the normal range. In 1 case of adrenal tumor, the initial effect of cortisone was to cause an increase in excretion of 17-ketosteroids from 95 to 120 mg. per twenty-four hours. On continued therapy the 17-ketosteroid excretion decreased to 40 mg. per twenty-four hours over a period of fifteen days. Operation was performed at this stage. ACTH was administered to 2 of the patients. There appeared to be a lowered response to ACTH, as judged by changes in 17-ketosteroid and corticoid excretion in the patient with adrenal tumor.

SATURDAY, JUNE 7, 1952

9:00 A.M.: PITUITARY HORMONES; THYROID

CHAIRMEN: J. W. CONN AND R. L. NOBLE

51. The Enhancing Effect of Somatotropin on the Mammary Growth Induced in Rats with Estrin, Progestin and Mammotropin.

W. R. Lyons, C. H. Li and *Ruth E. Johnson* (by invitation).

From the Divisions of Anatomy and Biochemistry, and the Institute of Experimental Biology, University of California, Berkeley, California.

In order to induce the change from a duct system to lobulo-alveolar development in an oophorectomized-hypophysectomized rat's mammary glands, 3 hormones have been found necessary: estrin, progestin and mammotropin (prolactin). However, this triad, at dose levels used to date, has not sufficed to bring about full lobulo-alveolar development equalling that obtained in oophorectomized, but not hypophysectomized rats, with estrin and progestin.

Our 2- to 3-month-old, hypophysectomized, oophorectomized rats consistently lost weight during the 10-day treatment with daily doses of estrone (10 I.U.), progesterone (4 I.U.) and mammotropin (60 I.U.), started immediately after operation. Despite weight losses ranging up to 30 grams, this treatment caused proliferation of mammary alveoli in the form of small lobules. By injecting purified somatotropin as well as these 3 hormones, the normal growth rate of such rats was restored at the 1.0-mg. daily dose level, or the usual weight loss prevented at the 0.1 mg. daily dose level. Even at the lower dose level, a definite enhancement of mammary growth was observed. Daily doses of somatotropin, from 0.1 mg. to 6.0 mg. given for ten days with estrone and progesterone but no mammotropin, failed to cause lobulo-alveolar development.

52. Interaction of Growth Hormone with Thyroxin and Other Hormones, as Measured by the Tibia Test.

Irving I. Geschwind and *Choh Hao Li.*

From the Hormone Research Laboratory, Department of Biochemistry, University of California, Berkeley, California.

The hypothesis is advanced that maintenance of the hypophysectomized animal by hormone replacement will enhance the sensitivity of such animals as assay subjects. The first series of experiments has involved the replacement of metabolic activity due to the thyroid, by means of thyroxin injection. The specific assay procedure investigated has been the tibia test of Evans, Simpson, Marx and Kibrick (*Endocrinology* **32**: 13, 1943) for the estimation of growth hormone (G.H.) potency.

Female rats of the Long-Evans strain were hypophysectomized at 28 days of age. Two micrograms of *dl*-thyroxin (Squibb) were injected subcutaneously once daily, beginning on the day of operation and continuing until sacrifice. Growth hormone preparations were injected intraperitoneally daily for four days beginning on the eleventh postoperative day. Autopsy of the animals took place twenty-four hours after the last G.H. injection. The following table summarizes the results obtained:

Treatment	No. of rats	Total dose, G.H.	11-Day body weight gain	15-Day body weight gain	Tibia width	G.H. equivalent*
		μg	Gm.	Gm.	micra	μg
Growth hormone	21	25	+ 4.7	+ 5.1	225	12
Growth hormone	14	50	− 1.1	+ 2.2	242	1?
Growth hormone +thyroxin	15	25	+ 6.1	+ 9.5	253	27
Growth hormone +thyroxin	16	50	+ 8.5	+11.2	273	48
Thyroxin	12	0	+13.3	+13.0	231	15
Control	36	0	+ 1.1	0.0	166	0

* From curve in Greenspan, Li, Simpson and Evans (*Endocrinology* **45:** 455, 1949).

The statistical significance of the effects of thyroxin has been determined. The ratio of potency of growth hormone in the thyroxin-treated animals to that in untreated animals is 2.09, with the range of potency in 95 cases out of 100 equal to 1.52–2.87. Furthermore, in the nine days of thyroxin treatment prior to growth hormone injections, treated animals gained 7 grams more than untreated animals. The effect on the tibia test of thyroxin alone, is highly significant.

The consequences of maintenance with ACTH, ACTH plus thyroxin, and DCA plus testosterone propionate are also presented, as are the effects of thyrotropic hormone and thyroidectomy.

53. Radioactive Growth Hormone Preparations.

Martin Sonenberg (by invitation), *William L. Money, Rulon W. Rawson* and *Albert S. Keston* (by invitation).

From the Memorial Cancer Center and New York University-Bellevue Medical Center, New York, N. Y.

A pituitary preparation with growth hormone activity labeled by iodination with I^{131} has been administered intracardially to normal rats. The biologic activity has been assayed in hypophysectomized rats.

The biologic activity of the labeled hormone preparations has been retained.

Significant concentrations of radioactivity have been observed in the pancreas, adrenal, thyroid, kidney, liver, and spleen after the injection of the labeled growth hormone preparation. The concentration of I^{131} in the pancreas was found to increase to a maximum one hour after injection and then decrease at a slower rate. In the adrenal the maximum concentration of radioactivity was noted ten minutes after the intracardiac injection of the labeled growth hormone preparation.

Control studies with I^{131} labeled iodide, I^{131} labeled serum albumin, and other pituitary

preparations labeled with radioiodine have failed to demonstrate the same concentration of radioactivity in the pancreas.

54. Significance of Changes in the Activity of Hepatic and Renal *l*-Glutamic Dehydrogenase Produced by Growth Hormone.

Oliver H. Gaebler and *James C. Mathies* (by invitation).

From the Edsel B. Ford Institute for Medical Research, Detroit, Michigan.

Since *l*-glutamic acid dehydrogenase is an important enzyme in the metabolism of amino acids, it appeared of interest to study changes in its activity in the liver and kidneys of animals in which protein catabolism was diminished by treatment with growth hormone. Hypophysectomized and normal groups of control and treated rats were pair-fed or fed *ad lib.* Treatment with large and small doses of growth hormone was employed.

Results were related to the manner of feeding and resulting rates of growth. During rapid growth, induced by administering large doses of growth hormone to hypophysectomized rats fed *ad lib.*, *l*-glumatic dehydrogenase activity did not increase proportionately in either kidney or liver, and the concentration in these organs therefore fell. When all groups were pair-fed against hypophysectomized controls, growth was more limited, even with large doses of growth hormone, and the fall in concentration of the enzyme was not observed. Similarly, no decrease occurred after small doses of growth hormone in hypophysectomized rats fed *ad lib.* Growth hormone may diminish the concentration of an enzyme quite indirectly by increasing the over-all rate of growth without influencing the rate of enzyme formation.

55. Influence of Adrenal Hormones on Thyroid Function in the Hypophysectomized Rat.

K. E. Paschkis, D. Epstein (by invitation), *A. Cantarow* and *G. Friedler* (by invitation).

From the Division of Endocrine and Cancer Research, Jefferson Medical College Philadelphia, Pa.

Recent experimental and clinical observations indicate that thyroid function is decreased following injection of cortisone or ACTH, or as a result of stress causing release of endogenous ACTH. It has been suggested that under these circumstances suppression of thyroid function is caused by diminished secretion of TSH from the pituitary gland.

However, a direct action of cortical hormone on the thyroid-thyrotropin mechanism, independent of pituitary secretion, has to be considered as a possible mode of action. In order to test this possibility, hypophysectomized rats were treated with TSH and with TSH plus ACTH or TSH plus cortisone, respectively. In these groups as well as in appropriate control groups the uptake of I^{131} was determined twenty-four hours after injection of a tracer dose (carrier-free). Treatment with TSH increased the uptake by the thyroids of hypophysectomized animals. Cortical hormone diminished the TSH-induced increased uptake. It is concluded, that cortical hormones act directly, and not necessarily through pituitary suppression, in inhibiting the TSH-thyroid mechanism.

56. Inhibition of Thyrotropin, Gonadotropins and Prolactin by ACTH.

Robert W. Brown (by invitation), *Dixon M. Woodbury* and *George Sayers.*

From the Departments of Pharmacology and of Obstetrics and Gynecology, University of Utah College of Medicine, Salt Lake City, Utah.

ACTH inhibits the action of growth hormone. Recent studies in this laboratory indi-

cate that ACTH, through mediation of the adrenal cortex, also exerts an inhibitory effect on the action of TSH, gonadotropins and prolactin. *TSH activity (percentage uptake of administered I^{131} by thyroid of hypophysectomized rat)*: controls, 21.1 ± 4.3; TSH (0.5 mg.), 70.9 ± 2.1; ACTH (5 U.S.P. units), 23.6 ± 3.3; TSH plus ACTH, 43.6 ± 2.1. *Gonadotropic activity (ovarian weight in mg. per 100 Gm. of hypophysectomized rat)*: controls, 22.0 ± 2.7; FSH-LH mixture (5 mg.), 58.3 ± 1.2; ACTH (40 U.S.P. units), 22.9 ± 2.4; FSH-LH mixture plus ACTH, 46.2 ± 3.7. *Prolactin activity (mM P-31 per Kg. of crop-sac, immature pigeon:* bioassay method described in *Proc. Soc. Exper. Biol. & Med.* **76:** 639, 1951): controls 71.4 ± 5.0; prolactin (1 mg.), 94.9 ± 1.1; ACTH (80 U.S.P. units), 70.2 ± 2.8; prolactin plus ACTH, 85.2 ± 1.5. Adrenalectomy abolishes the inhibitory effect of ACTH on growth, TSH and gonadotropic activities in hypophysectomized rats. Bioassay of adenohypophyseal hormones contaminated with relatively large amounts of ACTH should be conducted in hypophysectomized adrenalectomized animals. The data suggest that, in man, at least part of the action of an excess of cortical steroids in inducing thyroidal and gonadal dysfunction, is at a target-organ level.

57. Graves' Disease: Hyperthyroidism or Hyperpituitarism?

Sidney C. Werner, Howard Hamilton (by invitation) and *Martha Nemeth* (by invitation).

From Columbia University, College of Physicians and Surgeons, New York, N. Y.

The cause of Graves' disease is unknown. An endogenous thyrotropin excess from a pituitary acting to maintain an abnormally high blood level of thyroid hormone, or no longer responsive to the hormone, could cause hyperthyroidism. These possibilities have been tested. TSH (30 mg. for three days) was given to 21 thyrotoxic patients and increased SPI from 12.5 to 15.1γ per cent without a change in RAI uptake. After I^{131} therapy (14 patients) TSH increased SPI from 6.0 to 7.7γ per cent and RAI uptake from 31 to 38 per cent.

Desiccated thyroid (17 patients) did not suppress hyperthyroidism. With doses up to 0.6 Gm. daily for two to six weeks RAI uptake remained about 55 per cent; SPI rose from 12.1 to 12.7γ per cent. After I^{131} remission (17 patients), with a dose 0.12 Gm. daily, of RAI uptake changed from 46 to 35 per cent and SPI from 6.7 to 8.5γ per cent.

The findings do not correspond with those expected if hyperthyroidism were mediated through the pituitary. The occasional response seen after thyroid administration in active hyperthyroidism has been shown to be nonspecific. These data are presented, as well as control data from healthy subjects.

58. Serum Thyrotropic Hormone Bioassays in Patients Exhibiting Various Thyroid States.

Benjamin Simkin, Paul Starr and *Charles Hancock* (by invitation).

From the University of Southern California, School of Medicine, Los Angeles, California.

Thyrotropic hormone (TSH) bioassays of the sera of patients exhibiting various thyroid states were performed by a modification of D'Angelo's stasis tadpole method, in which the increase in height of the tadpole thyroidal acinar cells was the end-point for the assay. The mean acinar cell height (MACH) of 95 control tadpoles was 5.27 microns. No circulating TSH was detected in the sera of 11 normal euthyroid individuals (MACH = 5.31 microns), in the sera of 2 patients with panhypopituitarism (MACH = 5.20 microns), or in the sera of all but one of the patients with Graves' disease (MACH = 5.54

microns). Definitely detectable concentrations of TSH were found in the sera of all 8 patients with primary hypothyroidism (MACH = 6.64 microns); these results are statistically significant. Observations on the effect of desiccated thyroid on circulating levels of TSH in this last group of athyreotic patients were made. Small daily doses of desiccated thyroid (1/10–1/4 grain) either increased or did not suppress serum TSH levels. Larger daily doses of thyroid (1–2 grains) resulted in partial or complete suppression of serum TSH levels (MACH of group = 5.67 microns). Serial curves illustrating this have been made. Little or no TSH was detectable in the sera of exophthalmic patients (MACH = 5.71 microns). In this connection, the parenteral administration of thyroid stimulating doses of TSH to 13 patients, both with and without thyroid glands, for from three to twenty-two days, failed to cause any statistically significant increase in proptosis.

59. Life Stress and Thyroid Function in Human Subjects.

B. S. Hetzel, D. S. de la Haba and *L. E. Hinkle, Jr.* (introduced by Willard O. Thompson).

From the New York Hospital, Cornell Medical Center, New York, N. Y.

The plasma protein-bound iodine (PBI) has been shown to be a measure of the blood thyroid hormone and is being used for the diagnosis of thyroid disease. In this investigation hitherto unsuspected fluctuations have been found in association with exposure to stressful life experiences.

The alkaline ash method of Barker has been used and found to give satisfactory results, so that deviations of greater than ±12 per cent have been significant. Serial observation at hourly intervals have been made over 3-hour to 7-hour periods in euthyroid and hyperthyroid patients who had not previously ingested iodine in any significant amounts. Under neutral conditions the PBI has been observed to be remarkably constant during the period of observation in both euthyroid and hyperthyroid subjects. However, during experiences of stressful significance to the individual, marked fluctuations have been found—rises of as much as 100 per cent from one to three hours afterwards in some subjects, or falls of up to 60 per cent in others.

The pertinence of these findings to Graves' disease is currently under study. However, it is concluded that the possibility of these changes should be taken into account in using the PBI as an aid in clinical diagnosis.

60. Adrenal Cortical Response of Thiouracil-Treated Rats to Cold.

Henry H. Freedman (by invitation) and *Albert S. Gordon.*

From the Department of Biology, Washington Square College of Arts & Science, New York University, New York, N. Y.

Adrenal ascorbic acid and ester-cholesterol were followed in normal and thiouracil-treated rats exposed to a temperature of 5° C. In the controls, ascorbic acid decreased 33 per cent after one hour and returned to normal after twenty-four hours' exposure, all animals surviving. Ester-cholesterol concentration decreased by 62 per cent after eighteen hours' exposure. Following twelve weeks on 0.2 per cent thiouracil-feed, one hours' exposure to cold resulted in a 51 per cent depletion of ascorbic acid and no return to normal concentration; 75 per cent of the animals died within twenty-four hours. Ester-cholesterol was more greatly depleted after eighteen hours than in the normal group.

In animals clipped of body hair one hour before exposure, normals survived cold

eighteen hours or longer. Following four weeks on thiouracil, survival time was less than five hours. After four hours' exposure to cold, adrenal ascorbic acid was lower in the hypothyroid animals, than in a terminal state. Treatment with 0.5 ml. of Lipo-Adrenal Cortex/100 Gm./day for three days had no influence upon survival; 10 gamma of *d, l*-thyroxin/100 Gm./day allowed normal survival.

Thus the pituitary-adrenal axis of the thiouracil-treated rat responds to exposure to cold, and failure to survive does not appear to be caused by pituitary-adrenal impairment.

61. Effects of Temperature and Surgery Upon the Elimination of Radioiodine in the Rat.

*W. V. McFarlane.**

From the Department of Physiology, State University of New York, College of Medicine at New York City.

Rates of uptake and loss of I^{131} were estimated from the radiation passing the skin of 180-Gm. female Wistar rats and measured through a 1-cm. diameter portal. Rats kept at 30° C. lost 53 per cent of the initial count over the thyroid in twenty-four hours, whereas those at 1° C. lost 91 per cent. During the following six days the cold rats showed a consistent 25 per cent reduction in I^{131} level below the hot rats. Alternations of hot and cold environments produced corresponding retention and elimination of iodine. Excised thyroids showed the same order of difference between the two groups Thyroidectomy increased the elimination rate 10 per cent but the cold rats retained a 25 per cent greater elimination. Increased kidney and liver activity in the cold rats appeared to be the cause of this rate difference.

Since recent surgical wounds take up massive quantities of iodine and lose it more slowly than the normal tissues, *in vivo* measurements after operative procedures are distorted until healing is complete.

62. The Enterohepatic Circulation of Radiothyroxine.

A. Albert.

From the Mayo Clinic, Rochester, Minnesota.

Following intravenous injection of radio-*l*-thyroxine in rats, half of the radioactivity appeared in the liver and gastro-intestinal tract within one minute. During the first day, radioactivity in the liver fell while that in the small intestine and colon rose. After five days, 90 per cent of the radioactivity had been excreted in the urine and feces; of the material remaining in the body, twice as much radioactivity was present in the liver than in the rest of the carcass. Experiments with isolated portions of the gut indicated that radiothyroxine could be secreted directly from blood into the gastro-intestinal tract. However, the amount of radioactivity in the isolated small bowel was some six times greater when the bile duct was intact, than when it was ligated. Radioactive bile, collected from rats injected with radiothyroxine, was instilled into the duodenum. The radioactive material in the bile was readily absorbed, as attested by analysis of portal blood. The resulting distribution in tissues was similar to that found after intravenous radiothyroxine or after instillation of radiothyroxine into the duodenum. Results obtained with endogenously labeled thyroid hormone also support the concept that there exists a considerable enterohepatic circulation of radiothyroxine or iodine-bearing derivatives thereof.

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* Visiting Professor of Physiology, University of Queensland, Brisbane, Queensland, Australia.

63. Effect of Amphenone "B" on the Function of the Thyroid, Adrenals and Testes.

Robert H. Williams and *John R. Hogness* (by invitation).

From the Department of Medicine, University of Washington School of Medicine, Seattle, Washington.

The adrenomegalic and goitrogenic effects in rats of Amphenone "B", reported by Hertz *et al.*, are readily observed within ten days. During this interval there is a marked decrease in the capacity of the thyroid to concentrate I^{131}. The adrenals show a pronounced increase in the concentration and in the total quantity of cholesterol. There is eosinopenia and slight thymic atrophy. The testes show diminution in size.

The inhibitory effect on thyroid iodine concentration is evident within a few hours and is comparable in degree to that of thiouracil. In *in vitro* studies, this inhibition is observed whether the Amphenone has been given to the rat before thyroidectomy or whether it has been placed in the substrate of surviving slices of thyroid. Amphenone does not cause leeching of iodine from the thyroid. These observations suggest that Amphenone, like thiouracil, blocks an enzyme system concerned with the concentration of iodine in the thyroid.

The increased size and cholesterol concentration of the adrenal, along with eosinopenia and thymic atrophy, suggest that Amphenone causes an increased secretion of ACTH and adrenal corticoids. Whether there is proportionately as great an increased secretion of adrenal corticoids as there is increased ACTH is questionable; this is being investigated.

64. Acromegaly Followed by Panhypopituitarism.

W. O. Thompson, P. K. Thompson (by invitation) and *D. M. Mandernach* (by invitation).

From the University of Illinois College of Medicine, Chicago, Illinois.

In a 43-year-old man who had suffered from acromegaly for ten to twelve years there suddenly occurred a hemorrhage into the pituitary, with loss of vision and coma. Following operation, in which part of the pituitary gland was removed, hypothyroidism, hypoadrenalism and hypogonadism developed.

Evidence is presented for the presence of these deficiencies and a description of the favorable response to treatment with thyroid, cortisone and androgen.

2:00 P.M. JOINT SESSION WITH THE AMERICAN DIABETES ASSOCIATION

CHAIRMEN: ARTHUR R. COLWELL AND GREGORY PINCUS

65. Spontaneous Disappearance of Alloxan Diabetes in the Rat After Twelve to Twenty Months of the Disease.

Arnold Lazarow.

From the Department of Anatomy, Western Reserve University, Cleveland, Ohio.

Diabetic and subdiabetic rats have been used in this study. The latter were prepared by injecting a subthreshold dose of alloxan (30 mg./Kg., i.v.) and selecting those animals which showed normal blood sugar values (under 150 mg./100 cc.) and abnormal glucose tolerance curves. The rats were kept in metabolism cages in an air conditioned room, and the urine output, urine sugar, food intake, weight and blood sugar were determined several times a week. In the subdiabetic rats, glucose tolerance tests were performed at monthly intervals.

The most striking example of spontaneous disappearance of diabetes occurred in rat BB109. This animal was followed for twenty-seven months. During the first twelve months the blood sugar values ranged from 350 to 460 mg./100 cc. During the thirteenth and fourteenth months the rat gained weight, the blood sugar returned to normal (100–150 mg. per cent), and the polyuria, polydipsia, glycosuria, and polyphagia disappeared. Although the glucose tolerance was markedly decreased at sixteen months, there was a progressive improvement in the tolerance during the next six months; during the last six months of life the glucose tolerance curve was normal.

Of 14 diabetic rats which survived for over a year, 4 are still alive at thirteen to fifteen months; 1 of these has shown amelioration of the diabetes. One of 2 rats that died at thirteen months was just beginning to show amelioration at the time of death. Of 2 rats that died at fourteen to sixteen months, both showed normal blood sugar levels during the last two months of life. All of 6 animals that survived for twenty-two to thirty months showed normal blood sugar values for three to thirteen months prior to death.

Of 6 subdiabetic rats (normal blood sugar level but abnormal glucose tolerance) that survived for fourteen to twenty-eight months, 5 have shown an improvement in their glucose tolerance during the last two to twelve months of life. The unimproved rat is still alive at twenty months.

The possible explanation of these results (pancreatic beta-cell regeneration, exhaustion of the pituitary or the adrenal glands, etc.) is discussed.

66. Multiple Endocrine Adenomas: Report of 8 Cases Involving the Parathyroids, Pituitary and Pancreas.

Laurentius O. Underdahl, Lewis B. Woolner (by invitation) and *B. Marden Black* (by invitation).

From the Divisions of Medicine, Surgical Pathology, and Surgery, Mayo Clinic, Rochester, Minnesota.

Association of adenomas in the parathyroids and in either the pituitary or pancreas, or both, have been studied in 8 cases.

In 2 necropsy cases there were multiple adenomas of the islands of Langerhans and the parathyroid glands. In a third, a chromophobe adenoma of the pituitary, and multiple adenomas of the pancreas, parathyroids and adrenals were found.

In 4 of 5 surgical cases with chemical evidence of hyperparathyroidism, multiple parathyroid adenomas were found at operation. In the fifth, removal of one parathyroid tumor failed to eliminate hyperparathyroidism, so that existence of additional tumors is inferred. Roentgenographic evidence of *intrasellar tumors was observed in 3 of these patients:* one had clinical acromegaly, and another a pituitary adenoma, removed surgically. Two patients had no roentgenologic evidence of pituitary tumor. Surgical exploration of the pancreas was carried out in 3 patients, multiple adenomas being removed in 2. In the third no tumor was palpated in the pancreas at subtotal gastrectomy for duodenal ulcer, although the history strongly suggested hyperinsulinism. Functioning islet-cell adenomas are highly probable in the remaining 2 patients on the basis of the history and the fasting blood sugar values of 23 mg. and 30 mg. per 100 cc., respectively.

67. Some Further Studies on the Relationship of Adrenal Cortex Hormones to Experimental Diabetes.

Dwight J. Ingle.

From the Research Laboratories of the Upjohn Company, Kalamazoo, Michigan.

(Abstract not received.)

68. The Evidence for Acceleration of Neoglucogenesis from Fat by ACTH, Cortisone and Related Compounds.

Laurance W. Kinsell, George D. Michaels, Sheldon Margen, Lenore Boling, and *John W. Partridge,* with the technical assistance of Nancy Dawson, George Fukayama, Rosalie Greer, and Sadie Smyrl.

From the Institute for Metabolic Research of the Highland Alameda County Hospital, Oakland, California.

As previously reported, the administration of ACTH, cortisone, or compound F to fasting individuals and to human subjects receiving very high fat intake, results in partial or complete elimination of the hyperketonuria resulting from the fast, or from the high fat intake, respectively. It has been shown that this effect is not referable to decreased fat combustion; and further, that it is not significantly referable to the increased rate of utilization of ketones; therefore, it appears that the effect refers to a change in the catabolic pathway of fatty acid.

Diabetic patients, maintained on formula diets containing only fat and protein plus mineral and vitamin supplements, together with crystalline insulin in sufficient amount to prevent extreme hyperglycemia, after suitable control periods have received ACTH, cortisone, or compound F. Prior to, and during such hormone administration, blood levels of sugar and ketones, and urinary excretion of sugar, ketones, and nitrogen, and other entities have been determined. Initially, it was impossible to carry out studies for any significant period of time, because of the rapid development of hyperglycemia and dehydration. When this problem was controlled, it was found that in some of the diabetic patients so studied, D/N ratios were obtained which significantly exceeded 2.65, and on some occasions exceeded 6.25.

The possible interpretations of these data are discussed.

69. The Banting Memorial Lecture: Insulin.

Charles H. Best.

From the University of Toronto School of Medicine, Toronto, Canada.

70. Studies on Steroid Diabetes in Man.

John J. Bookman (by invitation), *Stanley R. Drachman* (by invitation), *Louis E. Schaefer* (by invitation) and *David Adlersberg.*

From the Mount Sinai Hospital, New York, N. Y.

Among hundreds of patients treated with cortisone and ACTH for their basic disease, in 5 there developed the clinical features of steroid diabetes. None presented glycosuria or hyperglycemia prior to the institution of this form of hormonal therapy; in all, the signs of diabetes disappeared with the discontinuation of treatment or with reduction of dosage to minimal maintenance levels, and remained so during a follow-up period ranging up to one and a half years.

The group as a whole presented all the known features of steroid diabetes, although each patient did not exhibit all the features. Doses of insulin up to 70 and 110 units respectively, failed to control the glycosuria in 2 patients. Ketonuria was observed in only 1 instance. There was a negative nitrogen balance in the 1 patient so studied, despite a high level of protein intake. The 17-ketosteroids were low prior to, and during ACTH therapy in another individual. All patients were predisposed to diabetes; 4 patients had a familial background of diabetes, the fifth had a transient glycosuria ten years prior to cortisone treatment and a mild but persistent impairment of glucose tolerance one year

after cessation of therapy. This indicates that a primary deficiency of insular function was essential for the precipitation of steroid diabetes. In only 1 patient did manifest features of diabetes develop after cortisone therapy; in the 4 others diabetes failed to develop during cortisone therapy, but did so during ACTH administration.

READ BY TITLE

71. The Clearance of Exogenous Estrogen from the Blood of Women with Normal and Complicated Pregnancies.

A. E. Rakoff and *J. R. Sosnowski* (by invitation).

From the Department of Obstetrics and Gynecology, and the Division of Endocrine and Cancer Research, Jefferson Medical College, Philadelphia, Pa.

The ability of pregnant women to clear estrogen from the blood was tested by assaying the serum estrogen before and at appropriate intervals following intravenous injection of 100 mg. of potassium estrone sulfate. Whole sera were assayed on castrated mice in a standardized modification of the Fluhmann method. In 11 women in varying months of normal gestation high titers attained at five minutes after injection (400–1,980 m.u./100 cc.) fell rapidly at one hour (198–800 m.u./100 cc.), further at three hours (66–330 m.u./100 cc.), with nearly complete clearance at five hours (40–264 m.u./100 cc.), and a return to pre-injection levels (20–100 m.u./100 cc.) in twenty-four hours. These results are compared with the clearance of endogenous estrogen following delivery. The clearance curves closely parallel those for normal nonpregnant women (*Fed. Proc.* **10:** 107, 1951).

Of 5 toxemic patients 3 had normal results from clearance tests, 1 showed marked delay, and another, slight delay. A severe diabetic in mid-pregnancy showed moderately delayed estrogen clearance. A near-term patient with hepatic cirrhosis had an abnormally low pre-injection serum estrogen level, approximately normal estrogen clearance, and good conjugation of estrogens and pregnanediol in the urine.

72. The Influence of Folic Acid Antagonists Upon the Accessory Sex Tissues of Male Rats.

J. B. Trunnell, F. T. Brayer (by invitation) and *J. Awapara* (by invitation).

From the University of Texas, M. D. Anderson Hospital for Cancer Research, Houston, Texas.

The folic acid antagonists aminopterin and 2,6:diaminopurine were shown, in a series of experiments, to augment the response of the accessory sex organs of castrated mature male rats to injections of testosterone propionate. To a lesser degree there was also observed greater weight of these tissues in antagonist-treated animals (both castrated and intact) which were not given testosterone propionate. Although not uniformly true for each of the four organs studied (ventral and dorsal prostates, seminal vesicles, and coagulating glands), there was a general tendency for these antagonists to interfere with the growth-inhibiting effect of alpha-estradiol. This effect was greatest in the central prostates and seminal vesicles. Comparison of these results with those obtained by other investigators suggests that the precise relationships between folic acid and testosterone or estradiol action may vary considerably as functions of relative dosage and of duration of treatment.

73. A Syndrome of Eunuchoidism with Spermatogenesis and Normal Urinary FSH.

E. Perry McCullagh and *John C. Beck* (by invitation).

From The Cleveland Clinic Foundation and the Frank E. Bunts Educational Institute, Cleveland, Ohio.

Four patients are described who had Leydig cell failure with eunuchoidism and at the same time maintained tubular activity with production of spermatozoa. From a clinical standpoint the outstanding feature of this syndrome is the fact that there may be eunuchism of moderate to very severe degree in men with normal or nearly normal-sized testes. Prior to any treatment testicular biopsies showed spermatozoa in the tubules. There was, however, a distinct hypoplasia of Leydig cells amounting almost to absence. Semen was obtained in 3 of the patients and showed sperm in all, but a normal count in only 1. Gynecomastia was present in 1 patient. Skeletal maturity was estimated in 2; it was retarded by 5 years in one (aged 21) and was mature in the other (aged 40).

The sella turcica was normal by x-ray examination in all. The urinary gonadotropins measured as FSH were normal in all. ICSH (LH) was not measurable by the prostate phosphatase test (Schaffenburg and McCullagh) in 2. Urinary 17-ketosteroids levels were low normal.

Treatment with large doses of chorionic gonadotropin produced clinical results closely comparable to those which would be expected from testosterone.

The pathologic physiology is believed to be a selective failure of ICSH production. Such a syndrome has been postulated but to our knowledge has not previously been described.

74. Turner's Syndrome with Anovarism—Case Report.

J. Chavez Montes.

Universidad Nac. de Mexico, Mexico City, D. F.

According to the latest bibliography on this subject, cases of anovarism are very rare and as far as is known this is the first case demonstrated by laparotomy in Mexico and one of the few diagnosed prior to surgical exploration. Onset: during adolescence. Somatic findings: short stature (1.39 meters), span greater than height, lower segment greater than upper, webbed neck, low hair line, cubitus valgus, genu valgum, non-united epiphysis, normal sella turcica, myxedematous skin, absence of axillary and pubic hair, infantile breasts and genitalia, primary amenorrhea, and retarded mentality. Laboratory findings: BMR −26 per cent; FSH 96 R.U.; 17 ketosteroids 9.5 mg. in twenty-four hours; estrogens less than 6 R.U.; vaginal smears I. At laparotomy: hypoplastic uterus and salpinx, and no evidence of ovaries. Treatment with estrogens, thyroid and growth hormone produced a great improvement of the general picture, *viz.* development of secondary sex characteristics, regular menses, gain in height, and mental alertness.

75. The Influence of Mixtures of Biologically Active Estrogens on Target Organ Response.

Robert C. Grauer, Eugene Cutuly and *Eleanor Saier* (by invitation).

From the Alleghany General Hospital, Pittsburgh, Pa.

Mixtures of crystalline estrone, estradiol and estriol were made in varying proportions in order to observe their effect on the uterine weight response of the immature rat. A total of 2,250 rats were used. Basic dose response curves were established for each of the estrogens and their relative potencies were compared to estrone. Estrone and estriol mixtures

were made on a comparable weight basis. Since estradiol is approximately twenty times more potent than estrone, the weight of estradiol employed was one-twentieth that of estrone. This gave a "gamma equivalent dose." All three estrogens were most biologically effective in the low dose ranges. Mixtures of the estrogens caused an increase in the uterine weight beyond the capabilities of the individual estrogens. Thus, 100 per cent estrone and estradiol individually gave a maximum uterine response of between 80 and 90 mg. When these two were combined in varying proportions the uterine weight response was augmented beyond the additive effect alone. For example, 75 per cent estrone plus 25 per cent estradiol gave a uterine weight response in excess of 125 mg. Varying proportions of these two estrogens gave similar augmentation. Comparable responses occurred when estriol was added to estrone. When a less active biologic hormone (estriol) was added to a more active biologic hormone (estrone or estradiol) enhancement of tissue response occurred. The reason for potentiation resulting from these mixtures is unexplained. Important practical implications are suggested.

76. The Effect of Progesterone, Estrogen and Cortisone Upon Androgen Secretion and Clinical Picture of the Andrenogenital Syndrome.

Herbert S. Kupperman, Rita Finkler and *Jay Burger* (by invitation).

From the Department of Endocrinology, Newark Clinical Group and Newark Beth Israel Hospital, Newark, N. J.

A 37-year-old female with long standing adrenogenital syndrome (twenty-two years) associated with primary amenorrhea and more recently with hyperglycemia requiring insulin therapy was treated consecutively with 100 mg. of progesterone (aqueous suspension) three times per week, conjugated natural estrogen-equine, 10 mg. b.i.d., and cortisone in doses of from 12.5 mg. to 100 mg. o.d. The control 17-ketosteroid excretion levels prior to therapy ranged from 60–90 mg. per twenty-four hours and was not significantly depressed by progesterone therapy. High doses of estrogens decreased the patient's 17-ketosteroid excretion to a low daily level of 27.3 mg. However, despite the depressed 17-ketosteroid excretion achieved with estrogens no menstrual period occurred during or after steroid therapy. Cortisone depressed the patient's 17-ketosteroid activity from an 80 mg./day control level to 9.9 mg. after one week of therapy. In determining the dose of cortisone necessary to achieve a normal excretion of 17-ketosteroid, it was found that 25 mg. of cortisone per day administered orally maintained a normal 17-ketosteroid excretion. On the other hand, 12.5 mg. of cortisone per day did not completely control abnormal excretion, and 37.5 mg. per day depressed androgenic urinary excretion toward the lower limits of normal. While on cortisone therapy the patient experienced three spontaneous regular menstrual periods associated with an increase in breast size, and decreased rate of growth of facial hair, accompanied by improvement in partial cephalic alopecia. No change in serum electrolytes and only mild variation in insulin requirements was noted while the patient was maintained upon cortisone therapy. The role of cortisone in controlling 17-ketosteroid excretion in the adrenogenital syndrome and in the normal female is discussed.

77. The Failure of Vitamin E to Alter Significantly the Menopausal Index in the Surgical or Natural Climacteric Patient.

Meyer Blatt (by invitation), *Hans Wiesbader* (by invitation) and *Herbert S. Kupperman.*

From the Departments of Therapeutics and Obstetrics and Gynecology, New York University College of Medicine; and the Obstetrical and Gynecological Service (III Division), Bellevue Hospital, New York, N. Y.

Vitamin E was evaluated in menopausal patients, employing the blind testing technique and utilizing the menopausal index (M.I.) as the principal mode of studying its efficacy. Observations were controlled by comparing the effectiveness of vitamin E, with placebo, phenobarbital, and thirteen different estrogenic compounds of both the steroidal and nonsteroidal derivatives. By the use of the M.I., which represents the numerical conversion factor of the severity of the menopausal symptoms, we were able to show by statistical analyses that vitamin E when administered in doses ranging from 50–150 mg. a day was no more effective than a placebo in controlling the eleven most common symptoms observed in the natural or surgical climacteric. Twenty-seven per cent of the patients receiving a placebo showed moderate to excellent results and this was very comparable to the figure obtained when vitamin E was employed. Phenobarbital gave a higher therapeutic ratio and proved to be either moderately or very effective in controlling the menopause in 48 per cent of our patients. However, neither the placebo, phenobarbital, nor vitamin E approached the effectiveness in the treatment of the menopause that was achieved with conjugated natural estrogens-equine and ethinyl estradiol, the two most active compounds used in our study. Data on the comparative efficacy of other estrogenic compounds are presented.

78. Evaluation of Estrogenic Compounds by the Menopausal and Amenorrhea Index.

Herbert S. Kupperman, Meyer Blatt (by invitation), *Hans Wiesbader* (by invitation) and *Sakiko Togashi* (by invitation).

From the Departments of Therapeutics and Obstetrics and Gynecology, New York University College of Medicine; and the Obstetrical and Gynecological Service (III Division), Bellevue Hospital, New York, N. Y.

A statistical method of analysis has been applied to the clinical evaluation of estrogens in order to provide a more exact methodology for the study of the comparative efficacy of estrogenic compounds in the human. One point of comparison was made by means of the menopausal index (M.I.). The M.I. represented the sum of the numerical conversion factors of the severity of the eleven most common menopausal symptoms observed in the patient after a surgical or natural climacteric. In compiling the M.I., vasomotor symptoms, paresthesia, insomnia, and nervousness were given a weighted factor. Thirteen different steroidal and nonsteroidal estrogenic compounds have been evaluated by this means; and to provide a greater objectivity the blind testing technique has been utilized to remove the possibility of subjective bias by the interrogating physician. The comparable efficacy of the various preparations was arrived at by a combined study of both the M.I. and clinical improvement. The clinical improvement was based upon the physician's own evaluation of the effectiveness of the compound. A second criterion of evaluation of the different estrogenic compounds was attained by the use of the amenorrhea index (A.I.). The A.I. was applied to patients with primary or secondary amenorrhea. These patients were primed for a period of three weeks with a daily dose of estrogen, two times that used in the treatment of the menopausal patient. On the twenty-second day, the patient was given 100 mg. of progesterone intramuscularly in a single injection. The A.I. was obtained by multiplying the extent of withdrawal bleeding noted after progesterone therapy by the quotient obtained by dividing the duration of bleeding days by the time of onset in days after the injection of progesterone. The A.I. has proved to be an objective means of statistically comparing the estrogenic efficacy of different steroidal and synthetic estrogens. The estrogenic compounds evaluated included derivatives of crystalline estradiol, estrone sulfate, and nonsteriodal forms, such as TACE, dinestrol, hexestrol, and stilbestrol. Ethinyl estradiol and conjugated natural

estrogen-equine compounds proved to be the most potent in the doses employed when evaluated by the M.I. and A.I.

79. The Nature and Extent of Estrogen Conjugation as Reflected in Human Pregnancy Urine.

Benjamin F. Stimmel.

From the Rees-Stealy Clinic Research Foundation, San Diego, California.

We have measured by our modified procedure (*Fed. Proc.* **9**: 235, 1950) for the fractionation and photometric estimation of urinary estrogens, the amounts of free and a) β-glucuronidase, b) phenolsulfatase, and c) hydrochloric acid hydrolyzable estrogens in human pregnancy urine. In the 15 twenty-four-hour urine specimens so far studied (collected during the second and third trimesters) there were never more than traces (less than 50 micrograms) of free estrogen. The major portions of the conjugated estrogens of all three (estrone, estradiol, and estriol) estrogenic fractions were β-glucuronidase hydrolyzable, and residual hydrochloric acid hydrolysis yielded total estrogen titers in close agreement with those obtained by initial acid hydrolysis. The phenolsulfatase hydrolyzable moiety did not constitute more than 20 per cent of the total conjugated estrogen in any fraction, and residual hydrochloric acid hydrolysis frequently yielded total estrogen titers much lower than those obtained by initial acid hydrolysis. Concomitant pregnanediol estimations on the neutral fractions of each urine failed to uncover any phenolsulfatase hydrolyzable moiety, and β-glucuronidase and hydrochloric acid hydrolyzable titers were in good agreement.

80. Testosterone Activity as Influenced by Esterification and Vehicle.

J. H. Leathem, C. H. Howell (by invitation), *N. Antonchek* (by invitation) and *A. F. St. Andre* (by invitation).

From Rutgers University, New Brunswick, N. J., and Ciba Pharmaceutical Products, Inc., Summit, N. J.

Interest in androgens with prolonged activity prompted a study of 15 testosterone esters. Immature male rats were injected subcutaneously with 5.0 mg. of steroid after a 3-week post-castration interval. Groups of 9-12 rats were killed at 5-day periods up to forty days after injection; seminal vesicle, ventral prostate and frequently levator ani weights served as the criterion of response. Esters of testosterone, in oil, such as phenylacetate, undecylenate, valerate, isovalerate, butyrate, isobutyrate, hendecanoate and cyclopentyl propionate provided a much greater and more prolonged stimulating action of the accessory sex organs than the propionate. Organ weight regression was not complete thirty days after injection in comparison with the fifteen-day period for testosterone propionate. Microcrystalline suspensions containing crystals of 50 microns or less enhanced the action of the propionate and phenylacetate but decreased the action of the valerate and cyclopentyl propionate. Suspensions containing crystals of 75 microns or less enhanced the action of the isobutyrate and isovalerate but further depressed the effectiveness of cyclopentyl propionate. In general, the levator ani weights were related to the androgenic stimulation of the seminal vesicles. These data indicate that several esters of testosterone are more effective than the propionate and that microcrystalline suspensions may increase or decrease the activity of the steroid, with crystal size a factor.

81. A Comparison of Several Steroids with "Anabolic" Activity.

Thomas H. McGavack, Sidney Pearson and *Jonas Weissberg.*
From the New York Medical College, Metropolitan Hospital Research Unit, New York, N. Y.

External balances were determined before, during and after the administration of several steroids, each at more than one level of dosage, during a "fore" period of four days, a "therapeutic" period varying from seven to twelve days, and a "recovery" period varying from four to eight days; a standardized diet was maintained in each instance. A nitrogen-retaining effect was observed in the order named: testosterone propionate (I), 17-methylandrostenediol (II), and dihydrotestosterone (III). Delta-4-androstenedione (IV) had no demonstrable nitrogen-retaining action in the dosages used. In general, sodium and chloride retention was most markedly favored by II and IV. Significant changes in potassium were produced by II only with the dosages employed. 17-Ketosteroid excretion was increased in all cases, most markedly during the administration of IV. The subjects on whom these studies were made were for the most part people of 50 years of age or over. The discussion directs itself to the appropriate uses of these hormones in clinical medicine.

82. Vaginal Smear Studies in Postmenopausal Women Following Varying Doses of Estrogens.

A. M. Shearman, Mildred Vogel and *Thomas H. McGavack.*
From the New York Medical College, Metropolitan Hospital Research Unit, New York, N. Y.

The changes in the vaginal smears of 170 postmenopausal women before and after single doses of estrogen have been correlated with the size of the dose, the age of the subject, the intensity of the effect, the duration of the action, and the period elapsing between administration of the drug and the first observable effect (lag-time). The estrogens employed included free estradiol, estradiol benzoate, estradiol cyclopentylpropionate and estrone administered by intramuscular injection in menstrua that included sesame oil, propylene glycol, water and peanut oil, and conjugated estrogens administered orally. There was no correlation between lag-time and either the age or the amount of hormone administered. There was a direct relationship between the size of the dose and the duration of responses in the vaginal epithelium. This was not influenced by the age of the subject or by the number of years postmenopausal. However, intensity of the follicular reaction varied inversely with the age of the subject at the time the observations were made. The periods of observation ranged from 96 hours to four weeks, the lag-time varying from 24 to 480 hours. Only in those subjects receiving the largest dosages did a high degree of cornification of the vaginal epithelium develop.

83. A Rapid Chorionic Gonadotropin Assay Method for Clinical Purposes.

Paul H. Fried and *A. E. Rakoff.*
From the Department of Obstetrics and Gynecology and the Division of Endocrine and Cancer Research, Jefferson Medical College and Hospital, Philadelphia, Pa.

The levels of chorionic gonadotropin were determined by bio-assay for the weekly intervals of normal gestation on 182 blood specimens obtained from 60 normal gravid women. Assays were carried out on 7 patients every two weeks from the fifth week of pregnancy to the postpartum period. The titers increased rapidly until the eighth week

of pregnancy (20,000 to 50,000 rat ovary hyperemia units per 100 cc. of blood) and then dropped slowly until the eighteenth week to a range (2,000 to 3,300 R.O.H.U.), which was maintained until delivery. The curves differed from those reported by others only by virtue of the higher titers and a smaller range for the normal levels. The latter were attributed to the increased sensitivity and accuracy of the end point, using the rat ovary hyperemia reaction enhanced by the addition of pituitary extract. A comparative study of various biologic pregnancy tests led to the present choice, despite certain disadvantages of the ovary hyperemia reaction; these, however, were overcome by the addition of the synergistic pituitary extract. The specificity, accuracy and sensitivity were demonstrated by assays of commercial chorionic gonadotropins and various pituitary extracts. The assay method has proved to be of aid in the diagnosis, prognosis and treatment of disturbances of pregnancy.

84. Further Studies on the Action and Effectiveness of Various Forms of Testosterone.

James T. Bradbury and *James Robert Hendon.*

From the Institute for Medical Research and the Department of Medicine, University of Louisville, Louisville, Ky.

Previous comparative studies on testosterone (aqueous suspension), testosterone propionate and testosterone cyclopentylpropionate (in oil) are reviewed briefly. In the present project these three compounds were all given in equivalent doses of testosterone to each of 13 adult male patients in a tuberculosis sanitarium. In addition to the studies of the duration of action of each compound as measured by the daily excretion of 17-ketosteroids, the effect on nitrogen retention was estimated by the duration of creatin retention which was induced while creatin was being administered orally. This period of nitrogen retention was also correlated with the weight gains and the clinical responses of the patients.

85. Germinal Cell Arrest and Germinal Cell "Hyperactivity" in Relation to the Theory of Utilization of Gonadotropins.

Glenn E. Mortimore (by invitation) and *Carl G. Heller.*

From the University of Oregon Medical School, Portland, Ore.

The sharp contrast in urinary gonadotropin titers encountered in two superficially similar types of defective spermatogenesis in oligospermic men supports the "utilization theory."

Elevated gonadotropins were encountered in 15 patients in whom testicular biopsies revealed varying degrees of *germinal cell arrest.* Gonadotropin titers averaged 73 as contrasted to our normal of 24. The gonadotropin elevation in this group in which germinal cell activity is clearly less than normal is consonant with lack of utilization.

No elevation in gonadotropins was noted in 11 patients in whom testicular biopsies revealed an abundance of all germinal elements including secondary spermatocytes, spermatids and spermatozoa. In contrast with the normal picture, the products of germinal cell activity continued in a disorganized way, beyond the ordinary limits, to occupy most of the lumen—thus, in effect, plugging the tubules. In contrast to germinal cell desquamation, no necrosis was found and no denuded areas noted. This lack of gonadotropin elevation (average 28) when spermatogenic activity is not diminished (as

attested morphologically by the superabundance of germinal cells), is in keeping with utilization of gonadotropins during active spermatogenesis.

86. The Hormonal Factor in Painful Telangiectasia in Pregnancy.

Paul H. Fried, Paul K. Perilstein (by invitation) and *Frederick B. Wagner, Jr.* (by invitation).

From the Department of Obstetrics and Gynecology, the Department of Medicine, the Department of Surgery, and the Division of Endocrine and Cancer Research, Jefferson Medical College and Hospital, Philadelphia, Pa.

A study of 26 pregnant patients with varicose veins indicated that the distress and debilitation due to pain in the lower extremities were caused in each case by disturbances in varicosities of conglomerate groups of superficial venules and veins, whereas changes in the varicosities of the saphenous vein system were associated with a sense of heaviness and tiredness. The involved venules had definite characteristic features amongst which were the site, histologic appearance, the association of symptoms with changes in the vessels, appearance early in pregnancy, and regression shortly after delivery. In view of these findings, levels of the circulating sex hormones of pregnancy were evaluated by hormone assays. Urinary pregnanediol, serum gonadotropin, and serum estrogen levels were normal in 10 asymptomatic patients. Similar assays were carried out in 16 women whose varicosities were associated with symptoms and signs. The serum gonadotropin levels were normal. With one exception the urinary pregnanediol and/or the serum estrogen titers were decreased. In 2 cases the steroid hormone levels were normal prior to, but lowered after, the onset of symptoms and signs. Excellent therapeutic results were obtained with large dosages of diethylstilbestrol in a small preliminary group of patients.

87. Testicular Damage in Rats Induced by Radioactive Phosphorus.

Clyde Biddulph, Robert H. Peterson (by invitation) and *A. Earl Johnson* (by invitation).

From the Division of Physiology, Utah State Agricultural College, Logan, Utah.

Adult male Sprague-Dawley rats weighing 300 to 400 grams were injected intraperitoneally with 0.031, 0.062, 0.125, 0.25, and 0.5 millicurie of P^{32}, and subcutaneously with 0.031, 0.125, and 0.5 millicurie. They were sacrificed at 1, 3, 5, 7, 14, 28, and 34 days after injection. The testes of both groups, and the liver, kidney, and adrenal cortex of the group injected intraperitoneally, were examined for histopathologic changes.

No changes that were attributable to the injected P^{32} were found in liver, kidney or adrenal cortex, but damage was found in seminiferous tubules of the testes of both groups. Greatest damage occurred 14 days after injecting the P^{32} intraperitoneally, and 28 days after injecting subcutaneously. The damaged tubules were considerably smaller than normal, had a rough, irregular basement membrane and were usually disorganized and filled with acellular material. In some tubules, spermatozoa were greatly reduced in numbers or completely absent, whereas the Sertoli cells were apparently undamaged. In some tubules, giant or bizarre cells having a round, dark-staining nucleus and numerous vacuoles were found. The observed damage in the testes was in general similar to reported radiation damage.

The testes of rats injected for 28 and 34 days were smaller than normal and contained considerable fluid, which escaped readily as soon as the tunica albuginea was cut.

88. Studies in Steroid Fluorescence.

Joseph W. Goldzieher.

From the Research Department, St. Clare's Hospital, New York, N. Y.

A variety of reactions known to produce fluorescence with steroids was studied on a number of biologically active or structurally interesting compounds. Correlations were sought between fluorescence spectra and molecular configuration. Phenolic steroids react most intensely; the conditions for obtaining maximum fluorescence were determined. The influence of changes in exciting radiation wavelength, and the quantitative aspects of the fluoresence spectra of these steroids and of urinary extracts were investigated. The data obtained indicate that the technique of measuring fluoresence must be revised for the determination of estrogen at low levels. On the basis of these spectral analyses, work is in progress to measure the individual components of estrogen mixtures by differential fluorometry.

89. The Use of Monobenzyl Ether of Stilbestrol in the Treatment of Chronic Anovulation and Amenorrhea.

Charles Lee Buxton.

From Columbia College of Physicians and Surgeons and The Sloane Hospital for Women, New York, N. Y.

Various ethers of stilbestrol have been found by Werthessen, Gargill and others to have an apparent stimulating effect on pituitary activity. The purpose of this paper is to investigate further the possible pituitary-stimulating or gonadotropic effect of the monobenzyl ether of stilbestrol by using it in the treatment of patients with chronic amenorrhea or anovulation who have no apparent cause for these abnormalities other than the lack of ovarian activity. Eighteen patients with the foregoing complaints were treated with this substance on 37 different occasions. The treatment consisted of the injection of either 2, 4 or 8 mg. of the monobenzyl ether every other day for a total of four injections, given in the case of the anovulatory cycle patients during the first part of the cycle. Before therapy was instituted these patients kept temperature charts for several months or more and in most instances had kept temperature charts for several years, as they were patients of long standing in the Sterility Clinic. These patients also had had one or more endometrial biopsies and all the cases of chronic amenorrhea had previously been treated by various techniques, including the production of menstruation by estrogen or estrogen plus progesterone. During the course of therapy these patients kept temperature charts, and following therapy in all instances endometrial biopsies were obtained. Of the 37 courses of therapy administered to these patients 13 were considered to have a successful result in terms either of the onset of menstruation in amenorrheic patients or the production of ovulation in anovulatory patients. The percentage of success in the cases treated is deemed sufficient to make further investigation of this product worth while.

90. A Clinical Study of Premenstrual Tension.

J. H. Morton, H. Additon (by invitation), *R. G. Addison* (by invitation) and *L. Hunt* (by invitation).

From the New York Medical College, Flower and Fifth Avenue Hospitals, New York, and The Westfield State Farm, Bedford Hills, N. Y.

Previous studies have indicated that premenstrual tension is the result of the un-

inhibited premenstrual rise in blood estrogen (Morton, J. H.; Premenstrual tension, *Am. J. Obst. & Gynec.*, **60**: 343–352, 1950). The present clinical investigation was undertaken at a state prison for women, to determine the incidence and severity of premenstrual symptoms; to correlate any possible connection between the time of commission of the crime for which the inmates were arrested and the phase in the menstrual cycles; and to evolve, if possible, an effective therapy for the relief of the premenstrual distress. About 400 volunteers were included in the study. Two hundred women were given a relatively high-protein, low-salt diet with supplementary feedings. Half of these women received ammonium chloride and vitamin B complex, and the other 100 women received a placebo. The remaining 200 were maintained on their regular diet without supplementary feedings but did receive the medication or the placebo.

Subjective symptoms were recorded in each instance before and after a course of therapy; daily weights were taken; work output and behavior patterns were noted before and after the study; and on a small group of volunteers, arbitrarily chosen, vaginal smears and glucose tolerance curves were obtained before and after therapy. Findings are recorded and conclusions drawn.

91. Masculinization of Female Rats Through Gravidic Hormones in the Presence or Absence of the Hypophysis.

Kitty Ponse (introduced by Hans Selye).

From the Institute of Experimental Biology, University of California, Berkeley, the University of Geneva, Switzerland, and the Institute of Experimental Medicine and Surgery, University of Montreal, Canada.

(This work will be published *in extenso* in Acta Endocrinologica Scandinavica in collaboration with H. Evans, M. Simpson and F. Carter.)

Whereas many investigators have injected gravidic hormones, attention was not focused on possible masculinization and on the respective roles of the ovaries and the pituitary on that reaction. Fifteen female rats hypophysectomized at one month were divided into 3 groups—uninjected controls; 20–40 R.U. daily PU (Antophysin, Winthrop, 560 units); 20 R.U. PMS (Gonadin, Cutter, 420 units)—from the third to the twenty-sixth day after operation. Fifteen rats served as non-hypophysectomized controls, 5 for each group. The clitoris of all injected females was virilized, but much more in hypophysectomized animals; primary follicle atresia favored development of hypophysectomized animals; primary follicle atresia favored development of hypertrophied theca-lutein cells instead of cystic or hemorragic follicles and corpora lutea. Glandular masses of hypertrophied theca-lutein cells seem, once more, responsible for virilization. The presence of ovaries is essential; the presence of the hypophysis not only unnecessary, but hindering. Adrenalectomy was performed to complete this study. Other target organs were examined (female ventral prostatas; male ones, grafted in the bursa ovarii; salivary glands). These experiments complete those performed in Geneva since the author discovered the possible virilization in 1932.

92. Renal Adjustments to Large Saline Loads in the Normal and in Diabetes Insipidus.

Agamemnon Despopoulos and *Andrew O. Fitzmorris* (introduced by Frederic C. Bartter).

From the United States Public Health Service Hospital, Baltimore, Md.

Renal and vascular responses of 4 normal subjects to an oral saline load (10 per cent of body weight in eight hours) were compared with those of a patient with diabetes

insipidus (DI). The solution contained Na, Cl, and HCO_3 in concentrations of 135, 100, and 35 mEq./L., respectively.

In normal subjects, approximately 30 per cent of the administered salt and water was excreted during the eight hours of loading. All showed increases in weight (3–5 Kg.), venous pressure (24–34 mm.), and plasma volume (1–2 L.). Augmented diuresis preceded natriuresis, but there was no hemoconcentration relative to Na or Cl. GFR increased 30–40 per cent in 3 subjects but remained unchanged in the fourth. No significant rise in RPF occurred. Within twenty-four hours all subjects had excreted 100 per cent of the test load.

In DI, 30 per cent of the administered salt and 100 per cent of the administered water was excreted during the eight hours of loading. Serum Na increased 11 mEq./L. GRF and RPF remained unchanged.

Of three possible renal mechanisms for increased salt excretion (decrease in fraction reabsorbed, increase in volume of filtrate, increase in salt concentrate), the first occurred in all subjects, the second in 3 normal subjects, and the third in the DI patient.

93. The Influence of Epinephrine on the Glycogen Content and Respiration of Lymphoid and Other Organs.

Fleur L. Strand (by invitation) and *Albert S. Gordon.*

From the Department of Biology, Washington Square College of Arts and Science, New York University, New York, N. Y.

Administration of a single dose of epinephrine (200 μg) to fasted male rats induces a shift in carbohydrate reserves over an observed period of from one to forty-eight hours. One hour after injection, there is a uniform drop in the glycogen content of lymph nodes, thymus, spleen, liver, muscle and blood, followed by an increase first of liver and blood glycogen at four hours, then of lymphoid and muscle glycogen at thirteen hours. Accompanying the increase in lymphoid and muscle glycogen is a secondary depletion of liver glycogen. These data suggest a reciprocal relation between liver glycogen and the lymphoid-muscle stores. By forty-eight hours glycogen levels return to normal. In the adrenalectomized rat, this response is altered only by the more rapid elevation of lymphoid and muscle glycogen, following initial depletion. The hypophysectomized animal is slower in replacing the lost glycogen.

Correlation of respiration findings (tetrazolium reduction) to glycogen levels demonstrates generally an inverse relation between these factors in the organs studied, except for the first hour after epinephrine injection.

The results indicate that the major effects of epinephrine on carbohydrate reserves are not mediated through cortical or pituitary hormones.

94. Metabolic and Therapeutic Effectiveness of Modified ACTH Preparations.

Peter H. Forsham, Harry Lee (by invitation), *Felix O. Kolb* (by invitation) and *Grant W. Liddle* (by invitation).

From the University of California School of Medicine, San Francisco, California.

ACTH preparations of varying molecular sizes were shown by Li and his associates to be devoid of adrenal growth-promoting properties, low in eosinopenic activity but of high potency as measured by adrenal ascorbic acid depletion in rats. The potency of the ACTH, expressed as international units based on ascorbic acid depletion, ranged up to 100 units per milligram of dry material. Such preparations were administered to human subjects under controlled conditions.

Using the complete ACTH preparation (Li, 1943) as a standard of comparison, the modified preparations, when given in doses of comparable ascorbic acid-depleting potency, were found to have definitely less potency when measured in terms of urinary hydrocortisone, eosinopenia, and therapeutic efficacy. These differences are represented in the following table:

	Original ACTH	Modified ACTH
Rat		
1) Eosinopenia	++++	±
2) AA depletion	++++	++++
3) Adrenal wt.	++++	0
Human		
1) Urinary "Cpd.F."	++++	++
2) Eosinopenia	++++	++
3) Clinical effect	++++	++

The routine assay of ACTH preparations using the ascorbic acid depletion method is discussed in terms of reliability and significance.

95. Adrenal Function in Chronic Pulmonary Tuberculosis.

Joseph W. Goldzieher and *James C. Edlin* (by invitation).

From the Tuberculosis Division, Manhattan General Hospital and the New York Medical College Metropolitan Hospital Research Unit, New York, N. Y.

The frequency, extent and importance of adrenal involvement in pulmonary tuberculosis and the role of the adrenal in the clinical symptomatology of this disease have been the subject of widely divergent opinions. Asthenia, hypotension, anorexia, weight loss and gastro-intestinal disturbances have been interpreted by some as indicative of adrenal insufficiency; others feel that these symptoms can be explained as direct manifestations of the disease itself. To gain further information, 68 patients were studied as to extent of disease, blood pressure, weight changes, sedimentation rate, cardiothoracic ratio, eosinophil count, 17-ketosteroid and neutral reducing corticoid excretion, and the results evaluated statistically. On this basis significant correlations were noticed between steroid excretion, sedimentation rate and weight changes. In patients with an elevated sedimentation rate in the acute and/or febrile phase, the 17-KS averaged 14.5–5.9, and the NRC, 2.9–0.99; those with a low sedimentation rate and minimal activity showed respective values of 18.6–7.0, and 3.5–1.05. Patients losing weight showed an average 17-KS excretion of 14.5–2.5 and NRC excretion of 2.6–0.65, whereas those maintaining weight averaged 17.6–6.8 and 3.3–1.2 respectively.

96. Desoxycorticosterone Trimethylacetate in the Treatment of Addison's Disease.

Dalton Jenkins (by invitation), *Walter L. Arons* (by invitation), *Thomas F. Frawley* (by invitation) and *George W. Thorn.*

From the Department of Medicine, Harvard Medical School and the Medical Clinic, Peter Bent Brigham Hospital, Boston, Mass.

A microcrystalline aqueous suspension of desoxycorticosterone trimethylacetate has been utilized in the treatment of 40 patients with Addison's disease; most patients have been followed for a period of from six to twelve months. A single intramuscular injection of 30 mg. (1.0 ml.) of the trimethyl derivative is effective for a period of from four to six weeks, approximately 1/30–1/45 of the total dose being absorbed daily.

Balance studies revealed significant sodium and chloride retention within forty-eight hours following injection of the hormone. Dosage is determined from the daily requirement of desoxycorticosterone acetate in oil. The optimum therapeutic dosage varied from 30 to 90 mg. monthly. Therapeutic response was evaluated by following changes in body weight, hematocrit, heart size (x-ray), blood pressure and serum electrolyte concentrations. Fluctuations in control associated with depletion of the hormone depot were avoided by the regular re-injection of hormone at 30-day intervals. The authors believe that this form of desoxycorticosterone administration supersedes all others presently available for the long-term regulation of electrolyte balance in patients with Addison's disease.

97. The Effect of Other Steroids on the Response to Cortisone.

Robert Gaunt, Constance Howell (by invitation) and *Nancy Antonchek* (by invitation).

From Ciba Pharmaceutical Products, Inc., Summit, N. J.

Cortisone acetate (1 mg. per day) was given to groups of 12 immature and adrenalectomized male rats for twenty days and the effect determined of adding DCA, sodium chloride, testosterone propionate (TP), and methylandrostenediol (MAD) to the treatment. The cortisone-inhibited growth rate was stimulated by DCA (1 mg. per day) and TP (.25 mg. per day), the two together being more effective than either alone. MAD (2 mg. per day), had no consistent effect on growth. Sodium chloride (1 per cent in drinking water), unlike DCA, did not antagonize cortisone-induced growth inhibition in either intact or adrenalectomized rats. Individual organs (weight expressed as Gm./100 Gm. body weight) responded as follows: (a) There was a somewhat variable tendency for all the steroids used to increase kidney and liver weight. (b) Testis weight was reduced by MAD and more so by TP. Cortisone increased relative testis weight in all cases and prevented in part the testis-inhibiting action of TP and MAD. (c) In the 1:8 dosage ratio used, TP was slightly more androgenic than MAD as judged by seminal vesicle weight, although TP inhibited the testis much more than MAD. Cortisone, despite its effect on testis weight, had no effect on seminal vesicle size. (d) Cortisone, as expected, caused profound adrenal atrophy, an effect seen in much milder form with DCA. The action of DCA and cortisone together was additive. TP or MAD alone did not influence adrenal size. The most striking action of MAD, however, was to prevent completely the adrenal atrophy expected after cortisone or cortisone plus DCA. TP had a similar but weaker action. (e) Both MAD and TP caused thymic atrophy, an effect exaggerated in both cases when given with cortisone.

When cortisone, 10 mg. per day, was given to adult rats, the loss of body weight and other extensive pathologic manifestations were, if anything, enhanced by adding 1 mg. per day of DCA to the regimen.

98. Nasal Administration of ACTH; Observations of Effectiveness Measured by Blood Eosinopenic Response.

Richmond W. Smith, Jr. (by invitation), *Laurie C. Dickson* (by invitation), *John B. Bryan* (by invitation) and *William D. Lowrie.*

From the Divisions of Metabolism and General Medicine, Henry Ford Hospital, Detroit, Michigan.

The effectiveness of nasal insufflation as a method of administering ACTH has been evaluated by observations of the blood eosinopenic response in 20 normal volunteers.

Powdered lyophilized ACTH (Armour) in doses of 10 or 20 mg. was delivered to the nasal membranes using the Armour posterior pituitary powder insufflator. Eosinophil counts were made in most instances at hourly intervals following the insufflation. In 50 experiments using 20 mg. in two divided dosages thirty minutes apart, the eosinopenic effect was significant at the second hour and maximal by the fourth hour. The percentage fall in eosinophils at the fourth hour averaged 58 for 18 individuals with normal control counts. By the sixth to seventh hours, the eosinophil counts approached the control levels. Subjective evidence of hypercortical response roughly paralleled the eosinopenic effect but often was clearly apparent as early as the first hour. Local effect on the nasal membranes varied. In most instances, it was characterized by a transient mild irritation or slight burning. Few experienced moderate lacrimation and/or rhinorrhea with no significant immediate or delayed effects. When finely powdered ACTH was insufflated slowly, the local symptoms were markedly reduced.

99. The Endocrine Pharmacodynamics of Renal Tubular Concentratability.

James T. Wortham and *Benjamin B. Wells* (introduced by H. N. Marvin).

From the Department of Medicine, University of Arkansas School of Medicine, Little Rock, Ark.

From physiologic consideration, Pitressin may be considered identical to the antidiuretic hormone (ADH) of the neurohypophysis. Disparity in degree of urine concentration following administration of 0.5 ml. of surgical Pitressin led to standardization of the Pitressin renal concentration test. By control of the test conditions during the two hours occupied by the test, constant and reproducible results in urine concentration were obtained in 10 physically normal psychotic females. Physical exercise nullified the Pitressin influence and there was evidence suggesting that this effect was produced through adrenal cortical activation.

Administration of several steroids was carried out prior to standardized Pitressin tests, at a time when the steroidal effect was calculated to be manifest upon renal tubules but before fluid compartment shifts could occur. Desoxycorticosterone acetate and cortisone produced a significant decrease in the Pitressin effect. Testosterone propionate and dehydroisoandrosterone were less effective but revealed a suggestion of activity.

It is concluded that, in the combination of influences which governs the ability of the renal tubules to concentrate, certain steroids may be antagonistic to the action of neurohypophysial ADH. Of the steroids tested, DCA is most active, and cortisone moderately active in this role.

100. Specific and Nonspecific Blocking Effects of Dibenamine Congeners on the Stress Response in Rats.

Charles H. Sawyer (introduced by Sidney Roberts).

From the School of Medicine, University of California at Los Angeles, and Veterans Administration Hospital, Long Beach, California.

Results of a study of some mechanisms by which stressing agents stimulate ACTH-release from the rat hypophysis revealed the following: 1) The eosinopenia characteristically observed one hour after a subcutaneous injection of epinephrine is prevented by the intravenous injection of the β-chlorethylamine adrenergic-blocking agent SKF-501, twenty-four hours earlier. 2) Dibenzylaminoethanol (D-ol), a related drug lacking adrenergic-blocking capacity, fails to prevent epinephrine eosinopenia. 3) Subcutaneous injection of formalin stimulates the same degree of eosinopenia at one hour as does

epinephrine. If the formalin stimulus were mediated by epinephrine, one would expect blockade of the mechanism by SKF-501 but not by D-ol. 4) However, both SKF-501 and D-ol are effective in blocking the formalin-induced eosinopenia. The nature of this blockade is unknown, but the condition does not develop in response to a generalized stress reaction to formalin twenty-four hours prior to a second formalin injection. Nevertheless, since D-ol prevents the effects of formalin on the ACTH-release mechanism without being capable of blocking epinephrine, the results fail to confirm the presence of a specific adrenergic mechanism in the "autonomic" phase of pituitary stimulation by formalin.

101. The Effect of ACTH, Cortisone and Surgical Stress on Urinary Gonadotropin Excretion.

Arthur R. Sohval (by invitation) and *Louis J. Soffer.*

From the Endocrine Laboratory and Clinic of the Medical Services, the Mount Sinai Hospital, New York, N. Y.

Urinary gonadotropin excretion was studied in two groups of patients. Group I comprises 22 subjects receiving ACTH or cortisone. In 9 cases an increased amount of urinary gonadotropin appeared within the first week. Group II consists of 18 patients who were subjected to surgical procedures under general anesthesia. In 8 cases an excessive quantity of urinary gonadotropin was demonstrated after operation. In each instance it appeared during the first four postoperative days and in 4 during the first twenty-four postoperative hours.

Since the assay method employed the uterine weight of immature mice, the possibility of a metrotropic rather than a gonadotropic substance in the urine of these patients was investigated. Such an action was excluded by assays of potent urinary extracts on spayed mice. That the increased urinary gonadotropic activity was due to excreted cortisone was considered unlikely, since 0.5 mg. of cortisone injected into immature mice five times during two and a half days caused no ovarian or uterine enlargement. The administration of 0.02 and 0.05 mg. respectively of epinephrine three times in twenty-four hours to immature mice exerted no gonadotropic effect. The nature of the gonadotropic substance in the urine of these patients remains unknown. It probably originates in the adrenal or in the adenohypophysis.

102. The Course of Parabiotic Hypertension in the Rat Following Adrenalectomy.

C. E. Hall and *O. Hall* (by invitation).

From the Department of Physiology, University of Texas Medical Branch, Galveston, Texas.

Immature littermate female rats were placed in parabiosis. In those pairs in which hypertension developed in one of the animals (Hall, C. E. and Hall, O.: *Texas Rep. Biol. & Med.* **9**: 714, 1951) the effect of adrenalectomy of the hypertensive animal or its normotensive parabiont on the course of the disease was studied. Total adrenalectomy of hypertensive rats, but not of their normotensive partners, invariably resulted in the blood pressure of the former returning to normal. A reappearance of hypertension resulted, although not necessarily to preoperative levels, if adrenal regeneration from a small fragment escaping removal at operation occurred. Animals studied for periods up to fifty-eight days postoperatively gave no indication of recurring hypertension. Adrenalectomy of the normotensive partner of a hypertensive animal did not have this effect. Since capillary anastomosis between the animals served to prevent adrenal insufficiency

from occurring in adrenalectomized rats in parabiosis with intact partners, but did not serve to maintain hypertension, it is concluded that adrenal glands are essential for maintenance of parabiotic hypertension and that return to normalcy of blood pressure in hypertensives is not a manifestation of the development of adrenal insufficiency.

103. The Assay of Antidiuretic Substances.

Marvin Stein (by invitation), *Robert Jinks* (by invitation) and *I. Arthur Mirsky*.
From the Department of Clinical Science, School of Medicine, University of Pittsburgh, Pittsburgh, Pa.

When rats are given a gavage of 5 ml. of 0.2 per cent NaCl per 100 Gm., which is repeated one hour later, the volume of urine excreted after the second gavage is linear on time for six minutes ($b=0.1148$ $P<0.001$). Accordingly, the volume excreted during the first twenty minutes after the gavage represents a constant fraction of the volume excreted at sixty minutes. The intraperitoneal injection of isotonic saline at the end of the first twenty minutes does not affect the subsequent rate of excretion, whereas the administration of an antidiuretic substance (ADS) reduces the rate of excretion so that the ratio: $\frac{\text{volume excreted at 60 min.}}{\text{volume excreted at 20 min.}}$ (R) is diminished. The regression of the reciprocal of R on the log of the dose of Pitressin is linear in the range of from 0.10 milliunit to 1.20 milliunits ($b=0.2684$ $P<0.001$).

When the total amount of NaCl administered minus the total volume excreted up to twenty minutes after the second gavage is designated as a "load," then the regression of the term: $1-\frac{\text{volume 60. min. after injection}}{\text{"load"}}\times 100$ (R_L) on the log of the dose of Pitressin administered at twenty minutes is also linear ($b=40.5229$ $P<0.001$).

In addition to the foregoing, assays were performed in accord with Bliss' design (1944) with 5 rats at each dosage level. When the response was expressed at 1/R, the standard error of the potency ratio was ± 30.27 per cent and the index of precision (λ) was 0.3287. When R_L was utilized to express the response, the standard error was ± 27.31 per cent and λ was 0.3199, but the slope of the log dose-response curve for individual assays showed greater variability than when R was utilized. The procedure reported herein for the assay of ADS is both sensitive and precise and permits the application of statistical design. (Reference—Bliss, C. I.: *Science* **100:** 577, 1944.)

104. Effect of Compounds B and F (Kendall) on Electroshock Threshold.

Arthur E. Heming, Dorsey E. Holtkamp (by invitation) and *Leo F. Mansor* (by invitation).

From the Research and Development Division, Smith, Kline, and French Laboratories, Philadelphia, Pa.

Cortisone acetate has been reported to lower the electroshock threshold (EST) of rats, whereas similar treatment with desoxycorticosterone acetate (DCA) resulted in an elevated EST (Woodbury and Sayer: *Proc. Soc. Exper. Biol. & Med.* **75:** 398, 1950). In the present study the foregoing results have been confirmed with a dose of 2 mg./rat/day and the effects of several other steroids on the EST have been studied. Desoxycorticosterone alcohol (DOC) (2 mg./rat/day), compound F acetate (2 mg./rat/day) and compound B alcohol (5 mg./rat/day) in an alcohol-saline vehicle were administered subcutaneously to groups of 4–6 bilaterally adrenalectomized, adult, male rats. By the tenth day of treatment, the mean percentage change in EST was +18.5 per cent and

+20 per cent in the DCA and DOC groups respectively, and was −8.5 per cent, −9 per cent, and 17 per cent in the groups treated with compounds F, B, and cortisone, respectively. It is apparent, therefore, that compounds B and F exert an effect on EST qualitatively similar to that of cortisone.

105. Studies in Radiosulfur Metabolism—Protein Metabolic Defect in the Nephrotic Syndrome; Modification with ACTH-Cortisone.

Sheldon Margen, Rosalie Greer (by invitation), *Nancy Dawson* (by invitation), *Harold Tarver* (by invitation) and *Laurance W. Kinsell.*

From the Institute for Metabolic Research of the Highland Alameda County Hospital, Oakland, California.

Two male patients with the nephrotic syndrome have been studied as follows:

1. Before treatment of their disease, the incorporation of S-35 into plasma proteins and the urinary excretion of the isotope were followed after a tracer dose of S-35 labeled methionine.

2. In the first patient, the S-35 study was repeated during ACTH-cortisone treatment, in the course of which the urinary protein excretion had been reduced from pretreatment levels of 16–20 Gm. per twenty-four hours to 2–3 Gm. per twenty-four hours by administration of ACTH and cortisone; a massive diuresis occurred.

3. In the second patient, the S-35 study was repeated after sufficient salt-poor human albumin had been administered to elevate the serum albumin level to 3.0 Gm. per 100 cc. (from the pretreatment level of 0.92 Gm. per 100 cc.). During this study, a complete remission was induced with ACTH.

From these studies one may conclude that in "nephrotics":

1. The rates of albumin synthesis are markedly accelerated as compared to the normal ($T^{1/2}2 = 1.4$ days—first patient; $T^{1/2} = 2.0$ days—second patient; $T^{1/2} = 21$ days—normal.)

2. The rate of globulin synthesis is not significantly abnormal.

3. ACTH and/or cortisone induce a rapid cessation or marked decrease in albuminuria.

4. ACTH and/or cortisone cause a marked reduction in albumin "turnover time."

5. ACTH and/or cortisone do not affect the rates of globulin "turnover."

6. The S-35 content of the urinary protein is indentical with that of the serum albumin.

106. The Catabolic Action of Thyroxine and Cortisone.

V. P. Hollander (by invitation), *C. D. West* (by invitation), *J. E. Rall* (by invitation) and *O. H. Pearson.*

From the Sloan-Kettering Institute, Memorial Hospital Cancer Center, New York, N. Y.

Intravenous *dl*-thyroxine was administered in a daily dosage of 2 milligrams for five days and 4 milligrams for five days to a 38-year-old male with chronic lymphatic leukemia who had marked lymphadenopathy, splenomegaly and hepatomegaly. A pronounced catabolic response was induced as evidenced by negative nitrogen and phosphorus balances and more than a 4-kilogram loss in body weight. The maximum catabolic effect occurred two days after thyroxine was stopped and subsided eight days later. Nitrogen and phosphorus were lost in the proportions to be anticipated if muscle tissue was destroyed; and, clinically, there was no apparent shrinkage of lymphoid

tumor masses. A spontaneous anabolic response followed the catabolic effect without apparent increase in tumor tissue.

Oral cortisone given for twenty-four days in increasing amounts up to 800 milligrams daily induced negative nitrogen and phosphorus balances and marked shrinkage of lymphoid tumor masses. Nitrogen and phosphorus were lost in proportions intermediate to the ratio of these elements in muscle and lymphoid tumor tissue, indicating that both types of tissue were destroyed.

Thus, thyroxine induced its catabolic effect predominantly on muscle tissue, whereas cortisone produced destruction of both muscle and lymphoid tumor tissue.

107. Effect of Estrogens and Androgens on the Serum Lipids and B-Lipoproteins in Men and Women.

S. J. Glass, H. Engelberg, R. Marcus and *J. Gofman.*

From the Cedars of Lebanon Hospital, Los Angeles, and the Department of Medical Physics, University of California, Berkeley, California.

In the past year we began a long-range study, by ultracentrifugal analysis, of the influence of the sex steroid hormones on the serum B-lipoproteins together with the total lipids, cholesterol, phospholipids and the cholesterol-phospholipid ratio. The object of these studies was to determine whether the sex steroid hormones played any role in determining the lower values of certain B-lipoproteins in the serum of normal women, compared with the values in normal men of similar age groups. To this end 16 men and 15 women (in the atherosclerogenic age groups) were given buccal preparations of estradiol and methyltestosterone, singly and combined, for periods varying from two to four months. Dosage was kept within physiologic limits, except in a few instances in which larger test doses were administered. No consistent changes in the direction of either a sustained increase or decrease in the serum lipids or B-lipoproteins followed these experimental trials with the single or combined hormones. These data, therefore, do not reveal any significant influence exerted by estrogens or androgens singly and combined on the serum lipids and serum B-lipoproteins. These findings are contrary to reports that estrogens do influence the cholesterol-phospholipid ratio.

108. Studies on the Role of the Thyroid in Changes in Blood Lipids Following Cortisone Administration.

Louis E. Schaefer (by invitation), *Stanley R. Drachman* (by invitation), *Chun-I Wang* (by invitation), *Solomon Silver* (by invitation) and *David Adlersberg.*

From the Mount Sinai Hospital, New York, N. Y.

In an attempt to establish the mechanism responsible for the elevation of serum lipids after prolonged administration of cortisone the following studies were performed:

1) Patients with completely nonfunctioning thyroids, as demonstrated by lack of I^{131} uptake after administration of a tracer dose, were studied during a control period of from four to eight weeks which was followed by a period of prolonged cortisone administration.

2) Dogs were observed through a period of normal thyroid function; they were then subjected to myxedema-producing doses of I^{131} until no significant I^{131} uptake was demonstrable in the thyroid, as measured directly over the gland; after stabilization of serum lipids they were given large doses of cortisone for prolonged periods.

3) Rabbits were investigated before and after complete surgical thyroidectomy; after serum lipids became stabilized they were treated with large doses of cortisone.

The analysis of serum lipids involved total and esterified serum cholesterol, phospholipids, total lipids and neutral fats. The results of these studies indicated the role of the thyroid in the regulation of serum lipid partition. The independent role of the adrenal cortex and the anterior pituitary in these mechanisms is discussed.

109. The Triad of Blue Sclerae, Brittle Bones, and Otosclerosis. A Hereditary Form of Osteogenesis Imperfecta.

Felix O. Kolb and *Howard L. Steinbach* (introduced by Peter H. Forsham).

From the University of California School of Medicine, San Francisco, California.

Members of two families were studied who showed through several generations all grades of the triad of blue sclerae, brittle bones, and otosclerosis (van der Hoeve's syndrome). Although a Mendelian dominant the "penetrance" was incomplete; several members escaped and only a few showed the complete triad. By x-ray examination and dental surveys, the transition was traced from the infantile form of thin, fragile and deformed bones to the disease in adults, devoid of fractures, but characterized by osteoporosis. At that stage the presence of blue sclerae, or otosclerosis, or a family history, may lead to the correct diagnosis in otherwise idiopathic osteoporosis.

The hereditary triad of osteogenesis imperfecta is to be differentiated from the congenital form, *i.e.*, osteogenesis imperfecta letalis of Vrolik, which practically never involves other family members. This type is characterized by multiple fractures, resulting in fetal death or a deformed infant with blue sclerae; survival to adulthood is rare. A typical instance diagnosed in utero is also presented.

A third more benign form of osteogenesis imperfecta exists, "osteopsathyrosis" of Lobstein, which shows only a hereditary tendency to fragile bones. It is limited to early infancy and childhood, but lacks blue sclerae and otosclerosis.

110. Serum Mucoprotein Level in Endocrine Diseases.

R. E. Mancini (by invitation) and *J. C. Garberi* (by invitation) (introduced by F. A. de la Balze).

Instituto Nacional de Endocrinologia, Godoy Cruz 1221, Buenos Aires, Argentina.

It is well known that various hormones may change the morphologic and histochemical composition (acid mucopolysaccharides and other polysaccharides) of connective tissue of certain organs. The authors felt it would be interesting to study in some endocrine disturbances, the level of a serum mucoprotein fraction detected by Winzler's method. They selected 65 untreated patients and 22 normal individuals and observed: a) No change in mucoprotein level in the following conditions—6 cases of simple goiter, 4 cases of hypogonadotropic hypogonadism, 3 cases of hypophyseal myxedema, 5 cases of acromegaly, 5 cases of mild myxedema of thyroid origin and 5 cases of posthyperthyroid exophthalmopathy. b) A small increase in 7 cases of adrenogenital syndrome. c) The highest level in 5 cases of Cushing's syndrome, d) A moderate decrease which rose to normal values after treatment in 10 cases of hyperthyroidism. e) A moderate increase, restored to normal values after treatment, in 7 cases of severe myxedema of thyroid origin. f) An unmodified level in 8 cases of Addison's disease, which showed a small decrease after treatment.

111. Endocrine Regulation of Serum Hexosamine Levels.

Norman F. Boas (by invitation), *Arthur W. Ludwig* (by invitation) and *Louis J. Soffer.*

From the Mount Sinai Hospital, New York, N. Y.

Serum hexosamine levels are shown to be elevated in a large variety of clinical diseases. Serial studies were performed to study the mechanism of the hexosamine changes. 1) Transient increases followed acute stress (myocardial infarctions (two) surgical operations (three)). 2) Equivocal increases followed epinephrine treatment of 2 normal males. 3) An increase occurred in a patient in Addisonian crisis. 4) ACTH and cortisone consistently lowered chronically elevated serum hexosamine levels (11 cases of disseminated lupus erythematosus and cases of rheumatic fever and rheumatoid arthritis). 5) Cortisone had no effect on 1 normal patient; ACTH caused a slight, transient rise and subsequent fall in 1 normal and in 4 hyperthyroid patients. Evidence that the serum hexosamine response occurred in the absence of the adrenals and was consistently suppressed by adrenal steroids suggested that the adrenal effect was secondary, acting on a basic hexosamine-increasing mechanism, also hormonal. 6) Testosterone in normal subjects (two) had no effect. 7) Patients with hyperthyroidism (five), exophthalmic goiter (five) and hypothyroidism (two) had normal levels. 8) A patient with Simmonds' disease had an abnormally low level. 9) A thyrotropic hormone fraction on the other hand caused very striking increases in 2 normal patients. Preliminary experimental data indicate that the stress-hexosamine response is augmented in thyroidectomized and adrenalectomized rats and abolished in the hypophysectomized rat. The significance of these data is discussed.

112. The Relationship Between Connective Tissue Mucopolysaccharide Content and Tissue Electrolyte Composition.

A. W. Ludwig (by invitation), *D. K. Chen* (by invitation) and *L. J. Soffer.*

From the Mount Sinai Hospital, New York, N. Y.

Experiments were performed to determine the effects of changes in the amounts of connective tissue mucinous ground substance upon the sodium, chloride, and potassium content and the hydration of that tissue. The combs of adult cocks and capons were analyzed for sodium, potassium, and chloride content. Total tissue water was determined. The hexosamine content of the tissue was utilized as an index of the amount of ground substance polysaccharide present. Total water content of the cock combs averaged 89.4 per cent $\pm$ 0.3, whereas that of the capon combs was 63.9 per cent $\pm$ 0.7. Sodium values ranged from 145 $\pm$ 2 mEq./Kg. of fresh tissue in the rooster to 82 $\pm$ 0.1 Gm./Kg. of whole tissue in the cock's comb and 2.0 $\pm$ 0.05 Gm./Kg. in the capon's. In terms of dry weight, these were 57.6 $\pm$ 2.2 Gm./Kg. and 6.8 $\pm$ 0.2 Gm./Kg., respectively. Calculation of the chloride space indicated that the increased water content of the cock's comb was entirely extracellular. The potassium content showed only slight changes.

Similar studies were carried out on the orbital tissues from normal and from exophthalmic guinea pigs. The results were similar, but less striking, possibly because of the greater cellularity of these tissues.

These data tend to bear out the hypothesis that the acid mucopolysaccharides of the ground substance act as ionized polymers (polyelectrolytes), and play an important role in determining the electrolyte composition and hydration of some tissues.

113. Effect of Thyroid Hormone and Thyrotropin on the Ground Substance and Connective Tissue.

J. Lester Gabrilove, Arthur W. Ludwig (by invitation) and *Louis J. Soffer.*
From the Mount Sinai Hospital, New York, N. Y.

The effects of thyroid hormone and thyroprotein (TSH) on the skin and subcutaneous tissue were studied in patients with primary and secondary hypothyroidism by means of serial punch biopsies. The specimens were stained with hematoxylin and eosin, toluidine blue, and colloidal iron (Rinehart) and examined histologically.

In subjects with primary (thyroidal) myxedema, there was a deposition of metachromatic staining material, presumably mucoprotein in nature. This substance differed from that encountered in normal skin in its more intense staining characteristics and in its localization of fibrillar aggregates between the collagenous fibers. The administration of thyroid hormone resulted in a quantitative decrease in this material. The metachromatic staining ground substance was also found in skin biopsy specimens taken from patients with Sheehan's syndrome. In these subjects, the material decreased in quantity following the administration of thyrotropin. Large quantities of this mucoprotein-like substance were present in lesions of localized myxedema.

The findings observed in hypothyroidism would suggest that the presence of thyrotropin is not essential for the deposition of this metachromatic staining material. As a consequence, the theory that the lesions observed in localized myxedema are due to thyrotropin may require revision.

114. A Rapid Procedure for the Determination of Serum Cholesterol.

Sidney Pearson, Sidney Stern (by invitation) and *Thomas H. McGavack.*
From the New York Medical College, Metropolitan Hospital Research Unit, New York, N. Y.

A simple direct procedure for the determination of total cholesterol in serum has been developed to meet the needs of a large-scale study of endocrinologic changes and lipid metabolism in old age. The procedure requires only the addition of reagents to serum and the measurements of the intensity of the resultant color. The determination is complete in less than thirty minutes. Sera were analyzed by this rapid procedure and by the classic Schoenheimer-Sperry method; the results obtained by the new method showed an average deviation of only 2.5 per cent from the results obtained by the Schoenheimer-Sperry method. The method makes possible the accurate rapid routine determination of large numbers of serum cholesterol values in clinical laboratories.

115. Hyperfunctional Endocrinopathies Treated with Toxins. Preliminary Report.

Manuel M. Villaverde.
Habana, Cuba.

It seems reasonable to use certain toxins to treat hyperfunctional endocrinopathies, for endocrines sometimes react hypofunctionally to the aggression of different infective agents, parasites, etc.

An acromegalic patient improved her condition after the injection of extract of *Necator americanus* (Dr. Federico Sotolongo, co-worker). She could not be infested by the parasite. (Extract: grind parasites to pulp; for each parasite, add 1 cc. of physiologic serum (phenol 5 per cent); filter.) She received 5 cc. daily or every other day (beginning with 0.1 cc., to known tolerance). After a few months both feet and hands were strik-

ingly reduced in size, and the countenance was notably smoother. The B.M.R. and the need for insulin were also reduced.

Two women with adrenal hypercorticalism received small amounts of tuberculin, either a commercial preparation, or the regular Koch's tuberculin up to 0.2 cc. per day. In the first case the B.M.R. was reduced from −8.5 per cent to −12 per cent, and the excretion of 17-KS from 12 to 9.0 and 8.87 mg./24 hours, but later increased to 11.12 mg. In the second case, the B.M.R. did not vary but the 17-KS decreased from 19.3 to 14.1 mg./24 hours.

Other patients with hyperparathyroidism are under treatment by *Monilia* toxin, but no conclusions have yet been reached.

116. A Proposed Ascorbic Acid Depletion Assay for ACTH, Which Correlates Clinical Effectiveness.

Joseph D. Fisher (by invitation) and *Robert E. Thompson.*

From the Armour Laboratories, Chicago, Illinois.

The Sayers assay for ACTH functions on the following assumptions: 1) the rate of response to intravenously administered ACTH and the rate of recovery is the same for all ACTH preparations, and 2) the only difference between preparations is the magnitude of response to the same dose. However, samples of equal potency as defined by the conditions of this technique may differ markedly in the duration of their action when injected subcutaneously and their effectiveness measured over the period of one to sixteen hours. Placement of preparations in a more viscous vehicle than saline (gelatin) results in the following: 1) greater magnitude of effect is produced with the same dose, and 2) duration of effect is strongly altered and greater differences appear between two preparations in gelatin than between the same two in saline vehicle. Use of saline vehicle, intravenous injection and one-hour duration may cause the potency to be under-estimated by from two to ten times as compared to results obtained by use of gelatin vehicle, subcutaneous injection and sixteen-hour duration. Use of saline vehicle in both techniques may result in a difference of as much as 50 per cent.There is evidence of good correlation between the duration assay and clinical response to intramuscular injection.

117. The Isolation of a Potential Adrenal Androgen from Human Urine After Administration of ACTH.

Ivan I. Salamon and *Konrad Dobriner.*

From the Sloan-Kettering Institute for Cancer Research, New York, N. Y.

From the urine of patients who received therapeutic amounts of ACTH, we have isolated 18β-hydroxy-Δ^4-androstene-3,17-dione, a steroid of adrenal origin, chemically related to adrenosterone in the same way that compound F is related to cortisone. This compound has not been found in the urine of normal subjects nor was its presence detected in the urine of patients treated with large amounts of cortisone. It would appear from the α-β-unsaturated ketone structure in ring A that this compound is secreted as a primary hormone by the adrenal gland and in part is excreted without tranformation to other metabolites. This substance may be an important constituent of the "adrenal androgens," and as such, play a significant role in the metabolic response to adrenal secretion. The identification of the compound will be described and the correlation with other adrenocortical metabolites will be discussed.

118. The Perfusion of the Isolated Human Adrenal Gland.

E. B. Romanoff (by invitation), *F. Ungar* (by invitation), *R. I. Dorfman* and *G. Pincus.*

From The Worcester Foundation for Experimental Biology, Shrewsbury, Mass.

Human adrenal glands taken shortly after adrenalectomy have been perfused with citrated whole blood and the perfusates analyzed for corticosteroid content prior to, and following ACTH administration to the preparation. Increases in total corticosteroid output measured as formaldehydogenic substances occur following ACTH administration, and recycling of this blood through the isolated gland usually results in a loss of the formaldehydogenic substance generated. The total steroid mixture is partially purified by chromatography with silica gel. When certain eluates, presumably concentrating the characteristic corticosteroids, are analyzed by paper chromatographic methods, the presence of a number of substances is indicated. Among these are compounds having the characteristic ketol side chain and others lacking this side chain. Certain compounds appear to possess the characteristic alpha, beta unsaturation of ring A, whereas others lack this configuration. With the possible exception of 17-hydroxycorticosterone (and that in only minor amount) no known corticosteroid has been identified. The administration of ACTH appears to increase the number of products obtained, but great variability in the type of product occurs from gland to gland and even from dose to dose of ACTH. Although a minor part of the observed variability may be due to technical considerations, other factors undoubtedly are involved, such as the state of the gland, the disease of the patient, and possibly the nutritional and hormonal regimen employed with the patient.

119. Resolution and Quantitative Analysis of Steroids in Adrenal-Vein Blood.

Max L. Sweat and *Gordon L. Farrell* (introduced by George Sayers).

From the Department of Pharmacology, University of Utah College of Medicine, Salt Lake City, Utah.

Plasma steroids are extracted with chloroform, partitioned between Skellysolve A and 80 per cent ethanol and chromatographed on a micro-silicic acid column. Ethanol-chloroform (0 to 5 per cent ethanol) chromatograms are analyzed by fluorescence, phenylhydrazine reaction and ultraviolet absorption. The column will resolve and quantitatively recover a mixture of 0.5 microgram each of E, F and B. A fluorescence technic has been developed which is sensitive to 0.25 microgram of F and of B (E does not fluoresce). The micro-column combined with fluorescence can quantitatively analyze fluorescent steroids in 0.2 ml. of plasma from adrenal-vein blood of the rat. The phenylhydrazine reaction is less sensitive and detects E, F and B; the absorption maximum for the phenyl-hydrazine derivatives of E and F is 400 mμ; for B and Q, 430 mμ. Adrenal plasma from intact rats (under stress) contains 2 to 4 micrograms of a B-like steroid, 2 to 4 micrograms of an unidentified steroid slightly more polar than B, and no detectable F. Peripheral blood from adrenalectomized rats, adrenal-vein blood from adrenalectomized rats, and adrenal-vein blood from hypophysectomized rats contain no detectable steroids. Each milliliter of adrenal-vein plasma of the dog (under stress) contains approximately 3 micrograms of an F-like steroid and 1 microgram of a B-like steroid. A third unidentified component, less polar than B, does not fluoresce but reacts with phenylhydrazine to give a maximum absorption at 400 mμ; it is present in an amount equivalent to 1 microgram of F.

120. Cortisone and Hydrocortisone as Precursors of Urinary Dehydroepiandrosterone.

Paul L. Munson, Alexis Labhart (by invitation) and *Peter H. Forsham.*

From the Biological Research Laboratories, Harvard School of Dental Medicine, The Medical Clinic, Peter Bent Brigham Hospital, and the Department of Medicine, Harvard Medical School, Boston, Mass.

The exclusive adrenal origin of dehydroepiandrosterone (dehydroisoandrosterone, DHA) is well established, but identity of the specific steroidal precursors has been unknown. Cortisone administration, for example, results in a marked decrease in urinary DHA (Munson and Horne), but this does not eliminate cortisone as a precursor of DHA, because its contribution to the titer of DHA may have been obscured by the lowered endogenous DHA accompanying cortisone-induced hypoandrenocorticalism. The problem is being investigated further during oral administration of cortisone and hydrocortisone (100 mg./day) to 3 men whose testes and adrenal glands were removed surgically for therapeutic reasons. Urinary DHA was determined in the neutral ketonic fraction by the method of Munson, *et al.* In the first subject, the maximum daily output of DHA was 2.1 mg. (40 per cent of 17-ketosteroids) during cortisone administration and 0.7 mg. (6 per cent of 17-ketosteroids) during hydrocortisone administration, as compared with 0.06 mg. during a brief period of no therapy. Thus, metabolic transformation of cortisone and hydrocortisone may be responsible, at least in part for the normal DHA content of urine. Nevertheless, because ACTH in intact men, stimulates so much DHA excretion (Landau, *et al.*), there still may be additional unknown adrenal steroids which are major precursors of DHA.

121. Correlation of Chromatographic Fractions of Urinary Corticosteroids with Adrenal Cortical Function After Cortisone Therapy.

Hildegard Wilson, Richard Fairbanks (by invitation) and *Joseph J. Bunim* (by invitation).

From the Department of Chemistry and Medicine and the Study Group on Rheumatic Diseases, New York University College of Medicine, New York, N. Y.

The increase in crude formaldehydogenic corticosteroid excretion values following the 48-hour ACTH test was not significantly different in cortisone-treated and untreated patients. Chromatographic methods did, however, reveal diminished corticosteroid excretion after cortisone administration. Extracts of acidified urine were chromatographed on paper and the $C_{21}O_5$ corticosteroids were eluted and assayed by the Porter-Silber reaction. One patient yielded 21 μg. per day before treatment, 225 μg. while receiving cortisone, and 6 μg. immediately afterwards. Another subject showed 60 μg. per day during cortisone administration, 12 μg. upon withdrawal, and 44 μg. subsequently. Crude formaldehydogenic assays did not reveal such differences.

During cortisone administration eluates identified as compounds F and E assayed 38 and 47 μg. per day respectively in one patient, and 137 and 61 μg. in the other. Similar amounts and proportions were observed in a man with Addison's disease receiving 150 mg. of cortisone daily, suggesting that the first 2 patients excreted chiefly metabolites of the administered cortisone. ACTH treatment gave strikingly higher absolute and relative amounts of compound F in the second patient. Thus, the quantitative pattern of individual corticosteroid excretion may be a good index of adrenal cortical function.

122. Human Corticosterone Metabolism. I. Corticosterone the Principal Precursor of Urinary Neutral Pettenkofer Chromogens (Dehydroepiandrosterone and Derivatives).

William Q. Wolfson, Mioko Eya (by invitation) and *William D. Robinson* (by invitation).

From the Rackham Arthritis Research Unit, University of Michigan Medical School, Ann Arbor, Michigan.

An increase in Pettenkofer chromogen (PC) excretion sufficient to elevate the PC-17-ketosteroid (KS) ratio is found after only three of the steroids which cause increased KS excretion: 1) dehydroepiandrosterone, itself the principal PC (Landau *et al.*), 2) corticosterone, and 3) 11-ketoprogesterone, a known corticosterone precursor (Haines). Corticosterone itself is not a PC nor does it increase apparent PC values if added to urine before hydrolysis. Neither bilateral adrenalectomy nor significant liver impairment prevents conversion of orally administered corticosterone to PC. Stimulation of Leydig cells with chorionic gonadotropin (Landau *et al.*) or administration of other steroids which are 17-ketosteroid precursors (*e.g.*, the common androgens, cortisone, hydrocortisone) all fail to evoke an increased PC output proportionate to KS output; hence PC-KS ratio falls. The PC-KS ratio likewise is abnormally low in the totally adrenalectomized cortisone-maintained patient, and PC output shows only trifling increases after an additional 100 mg. of cortisone or hydrocortisone. ACTH evokes proportionate increases in PC and KS output with little overall change in PC-KS ratio. All the foregoing changes occur in both crude and ketonic neutral fractions. Since only corticosterone induces a selective increase in neutral PC excretion when given in physiologic dosage, corticosterone is probably the major precursor of urinary dehydroepiandrosterone.

123. Human Corticosterone Metabolism. II. Evidence for Simultaneous Corticosterone and Hydrocortisone Secretion by Stimulated Normal Human Adrenals.

William Q. Wolfson, William D. Robinson (by invitation), *Aletha Hemmerline* (by invitation) and *Mioko Eya* (by invitation).

From the Rackham Arthritis Research Unit, University of Michigan Medical School, Ann Arbor, Michigan.

An increased rate of hydrocortisone (F) secretion can explain many actions of ACTH in man. However, at least two ACTH effects cannot be reproduced by F or cortisone (E) alone. 1) ACTH induces proportionate increases in urinary Pettenkofer chromogen (PC) and 17-ketosteroid (KS) with little change in PC/KS. F and E decrease PC/KS ratio by causing considerable increase in KS output without proportionately increased PC. Corticosterone (B) sharply increases PC/KS ratio by inducing increased PC excretion without proportionately increased KS output. Oral administration of suitable mixtures of F and B reproduces ACTH-induced alterations in PC, KS, and PC/KS ratio including certain fine qualitative details of the response to ACTH. 2) Salivary K/Na ratio is promptly elevated by either 30 units/day of high potency corticotropin or 400 mg./day of oral B. No significant change follows 200 mg./day of oral F. ACTH-induced salivary electrolyte changes are reproduced by mixtures of F and B which also reproduce ACTH-induced changes in urinary steroids. These findings suggest that corticotropin stimulation induces simultaneous secretion of corticosterone and hydrocortisone from the intact normal human adrenal cortex. They also illustrate a possible clinical technique for estimating the human F/B secretory ratio.

124. The Urinary Excretion of Alpha-Ketolic Metabolites of Adrenocortical Hormones Studied by Paper Chromatography.

E. M. Richardson (by invitation), *F. C. Dohan* and *James H. Jones* (by invitation).

From the University of Pennsylvania, Philadelphia, Pa.

Acetates of cortisone, hydrocortisone, 11-desoxycorticosterone, and 17-hydroxy-11-desoxycorticosterone were administered to patients with primary or secondary adrenocortical insufficiency. Hydrolysis of urinary steroids with glucuronidase was followed by acid during chloroform extraction. Paper chromatography was performed by a modification of the methods of Zaffaroni, Burton, and Keutmann, and of Tennent, Whitla, and Florey. This permits the use of a small aliquot of the 24-hour urine collection for the detection (with blue tetrazolium) and semi-quantitative measurement of steroids containing the alpha-ketol group. Identification of steroids, in general, followed the technic of Zaffaroni, *et al.*

Cortisone or hydrocortisone administration was associated with marked increase in the excretion of compounds having the characteristics of tetrahydrocortisone and an associated more polar steroid (probably tetra-hydro-hydrocortisone), as well as the appearance, in some instances, of alpha-ketols at the hydrocortisone and cortisone position. Only questionable increase in "tetrahydrocortisone" and "tetrahydro-hydrocortisone" resulted from the administration of 11-desoxycorticosterone or 17-hydroxy-11-desoxycorticosterone. The former compound produced an alpha-ketolic steroid at the hydrocortisone position (not hydrocortisone) and the latter produced large amounts of material almost at the cortisone position (not cortisone). In addition, 11-desoxycorticosterone produced large amounts of a rapidly moving alpha-ketol. Identification studies are in progress.

125. 17-Ketosteroid and Androgen Excretion in Orchidectomized Patients.

John C. Laidlaw (by invitation), *Frederick C. Goetz* (by invitation), *Dalton Jenkins* (by invitation), *Paul L. Munson, J. Hartwell Harrison* (by invitation) and *George W. Thorn.*

From the Departments of Medicine and Surgery, Harvard Medical School, The Harvard School of Dental Medicine, and the Medical and Surgical Services, Peter Bent Brigham Hospital, Boston, Mass.

It is known that, despite the removal of all adrenal and testicular tissue in patients with metastatic carcinoma of the prostate, 17-ketosteroids continue to be excreted in the urine (Huggins). We have performed complete bilateral adrenalectomy in 3 such patients whose disease had become resistant to control by orchidectomy and estrogen treatment. Following operation the patients were maintained on intramuscular DOC and cortisone or hydrocortisone by mouth. Intravenous ACTH tests showed no evidence of functioning adrenal tissue. Measurements were made of urinary 17-ketosteroids by a modified Holtorff and Koch procedure, and of urinary androgens by the chick-comb method of Rakoff. When no cortisone or compound F was given, the ketosteroids ranged from 1.7 to 6.1 mg. per day. During administration of 25 mg. of oral cortisone per day the ketosteroids were only slightly increased, whereas on 100 mg. of cortisone they were nearly double in value. The highest level of ketosteroids, 16.7 mg./day, was attained in a patient receiving 100 mg. of compound F. When only DOC was given, no urinary androgen was detected. Following 100 mg. of either cortisone or compound F, however, appreciable amounts of androgen were found. The therapeutic implications of these findings are discussed.

126. An In Vitro Effect of ACTH.

R. Haynes (by invitation), *K. Savard* (by invitation) and *R. I. Dorfman.*

From The Worcester Foundation for Experimental Biology, Shrewsbury, Mass.

Tissue slices from beef and pork adrenal cortices were incubated in blood in the presence of carboxy C^{14}-labeled sodium acetate. After incubation, the cortical hormones of the slices and the blood were concentrated by a combination of extraction, silica gel chromatography, and paper strip chromatography. After repeated paper strip chromatography, the radioactivity was found to be concentrated in the 17-hydroxycorticosterone zone. A portion of the 17-hydrocorticosterone which had been chromatographed three times on paper was oxidized with chromic acid. From this oxidation, a compound was obtained which migrated as adrenosterone and which was radioactive. Slices incubated with ACTH (Astwood preparation) yielded significantly higher radioactivity in the 17-hydroxycorticosterone zones of paper chromatograms than did slices incubated without ACTH. This difference in radioactivity persisted after the material in the 17-hydroxycorticosterone zone was eluted and rechromatographed on paper strips a second and third time. This suggests ACTH acted in this system to increase the rate of synthesis of cortical hormones in the tissue slices.

127. Effect of Surgery and Adrenal Stimulation on the Excretion of Pregnanediol-like Material in Men.

Bernard Zimmermann and *Amelia J. Wojack* (by invitation).

From the Department of Surgery, University of Minnesota, Minneapolis, Minn.

The fact that a significant portion of administered 11-desoxycorticosterone apparently undergoes conversion to pregnanediol glucuronide suggested that the urinary excretion of the latter substance might be utilized as an index of the endogenous production of 11-desoxycorticoids.

In the present study, in which male subjects were utilized, the increased excretion of pregnanediol or "pregnanediol-like" material (method of Guterman, modified by Hoyt and Levine) following administration of desoxycorticosterone acetate was confirmed. Furthermore, twofold to fivefold increases over baseline excretion were observed following major surgical procedures, under which circumstances the daily pregnanediol excretion ran parallel to eosinopenia and formaldehydogenic corticoid excretion. Similar results were obtained following ACTH administration in male subjects. Cortisone administration, on the other hand, in amounts adequate to cause eosinopenia and high formaldehydogenic steroid excretion, resulted in no elevation of pregnanediol output.

The pregnanediol excreted in these subjects appears to be the end-product of substances produced as a result of surgical stress and adrenal stimulation, which are metabolized differently from the oxycorticoids. The likelihood that these precursors are 11-desoxycorticosteroids and the relationship of this finding to theories concerning regulation of mineralocorticoids and post-surgical sodium retention are discussed.

128. Estimation of Conjugated Corticosteroids in Urine.

William J. Reddy (by invitation), *Peter H. Forsham* and *Dalton Jenkins* (by invitation).

From the Department of Medicine, Harvard Medical School, and the Medical Clinic, Peter Bent Brigham Hospital, Boston, Mass.

It has been shown that cortisone and related 17,21-dihydroxy-20-ketosteroids give a

relatively specific yellow color when reacting with phenylhydrazine in strong sulfuric acid at 60° C. (Porter and Silber). We have adapted this reaction to the estimation of the conjugated forms of cortisone and related steroids in urine. Free steroids are obtained by extraction with chloroform, following which conjugated steroids are obtained by N-butanol extraction. The naturally occurring conjugates appear to give the same type of color reaction obtained with free cortisone or cortisone acetate. The procedure may be carried out on small volumes of urine and in a relatively short time. Using pure cortisone as a reference standard, the following 24-hour values were obtained on a small group of patients and normal subjects: normal males, 4–12 mg.; normal females, 1–5 mg.; individuals receiving daily intravenous ACTH, 25–45 mg.; subjects receiving 500 mg. of oral cortisone per day, 50–130 mg.; a patient with Cushing's syndrome, 81 mg.; after subtotal adrenalectomy and receiving a maintenance dose of 25 mg. of cortisone per day, 3.3 mg.; 2 patients with Addison's disease, less than 0.05 mg. The practical implications of this procedure are discussed.

129. The Effect of Perchlorate on the Human Thyroid Gland.

James B. Wyngaarden (by invitation) and *J. B. Stanbury.*

From the Massachusetts General Hospital, Boston, Mass.

Thiocyanate prevents the accumulation of iodide by the thyroid gland of man and rat. The administration of a suitable dose of thiocyanate to a subject receiving thiouracil or a related drug causes a discharge of accumulated iodide from the thyroid gland. The mechanism for this action is not clear, but may be competition for receptor groups within the thyroid cell. A survey of other anions which might have similar activity has revealed the fact that perchlorate ion is approximately ten times as effective as thiocyanate in preventing the accumulation and causing the discharge of accumulated I^{131} from the thyroids of rats receiving propylthiouracil (Wyngaarden *et al.*, in press). It became of interest, therefore, to extend these studies to man. A series of thyrotoxic patients has been selected and it has been shown that perchlorate in small doses is capable of preventing the accumulation of tracer doses of I^{131} in the thyroid glands of these patients. If, on the other hand, these patients receive prior to the administration of the tracer dose of I^{131}, a goitrogenic drug, 1-methyl-2-mercaptoimidazole, administration of 100 milligrams or less of potassium perchlorate will cause a prompt release of the trapped I^{131} from the gland. The duration of the block created by the potassium perchlorate is several hours, and the effectiveness of the salt and the duration is such that it could conceivably be used therapeutically in man.

130. Protection by Somatotropic Hormone (STH) Against Experimental Tuberculosis.

Paul Lemonde (by invitation), *Maurice Panisset* (by invitation), *Maria Dobija* (by invitation) and *Hans Selye.*

Institut de Medecine et de Chirurgie experimentales and Institut de Microbiologie, University of Montreal, Montreal, Canada.

It has been demonstrated that cortisone sensitizes an otherwise resistant species, such as the rat, to infection by tuberculosis (Selye: First Annual Report on Stress. Montreal, Acta, Inc., 1951). We confirmed this fact in experiments on male Wistar albino rats (average body weight 170 Gm.), which received 3–5 mg. of cortisone acetate daily and were infected by intraperitoneal inoculation with 2 mg. (wet weight) of H37Rv human-type tuberculosis bacilli. Simultaneous treatment with 4–5 mg. per day of STH (Armour Lot No. 285-110) exerted a pronounced protective effect under these experimental con-

ditions. It may be concluded that the sensitivity of an animal to infection by tuberculosis depends largely upon hormonal factors. Preliminary experiments on mice infected with the same human-type tuberculosis bacilli suggest that, in a normally tuberculosis-sensitive species, STH exerts a protective effect, even if the animal is not specifically sensitized by cortisone.

131. Protection by Hypophysectomy Against Some of the Toxic Effects of Corticoid Hormone Overdosage.

Ernesto Salgado (by invitation) and *Hans Selye*.

Institut de Medecine et de Chirurgie experimentales, University of Montreal, Montreal, Canada.

Male albino rats (average body weight 100 Gm.) were sensitized to the toxic effects of mineralocorticoids by unilateral nephrectomy and 1 per cent NaCl (as drinking fluid); then they were treated with 10 mg. of desoxycorticosterone acetate (DCA) daily during twenty-one days. As expected, they exhibited myocarditis, nephrosclerosis, hypertension and polyuria. Simultaneous administration of cortisone acetate (1 mg. per day) aggravated the renal lesions caused by DCA, but remained without pronounced effect upon the other changes mentioned. In similarly sensitized hypophysectomized rats, DCA, or DCA plus cortisone, produced little or no nephrosclerosis and myocarditis, yet the blood pressure and diuresis were significantly augmented. These findings confirm and extend earlier observations (Selye: *J. Clin. Endocrinol.* **6**: 117, 1946; Stress. Montreal, Acta, Inc., 1950), suggesting that some of the toxic effects of corticoids (*e.g.*, myocarditis, nephrosclerosis) depend upon the simultaneous presence in the organism of anterior pituitary principle(s), presumably somatotropin and/or thyrotropin, whereas other effects (*e.g.*, hypertension, polyuria) are largely independent of the hypophysis.

132. Pluriglandular Insufficiency Without Panhypopituitarism.

R. Palmer Howard, E. S. Mills (by invitation) and *W. H. Mathews* (by invitation).

From the Oklahoma Medical Research Institute, Oklahoma City, Oklahoma, and Montreal General Hospital, Montreal, Canada.

Repeated studies of adrenocortical function were made in a 43-year-old diabetic male in whom marked hypothyroidism had recently developed. The urinary excretion of 17-ketosteroids and glucocorticoids, the serum electrolyte concentrations, and the response of circulating eosinophils to ACTH indicated hypofunction of the adrenal cortex both before and during treatment with thyroid substance. The diagnosis of panhypopituitarism was considered but eliminated by the presence of gonadotropins in the urine and of a certain amount of 17-ketosteroids, indicating some degree of hypophyseal and of gonadal function. Subsequent postmortem examination revealed marked atrophy of the thyroid, but grossly and histogically normal adrenal glands, and no significant abnormality in the hypophysis or the testes. In conclusion, 1) thyroid insufficiency may decrease the function of the adrenal cortex without greatly altering the function of the hypophysis or the testes; 2) thyroid therapy may not be accompanied by prompt restoration of adrenocortical function; and 3) hypofunction may exist without the accompaniment of detectable histologic abnormalities in the adrenal cortex.

The relationship of the pituitary, the testis and the pancreas to the hypofunctional state of the thyroid and the adrenal glands is discussed.

133. Polyostotic Fibrous Dysplasia with Sexual Precocity and Pigmentation (Albright's Syndrome) and Associated Non-neoplastic Acromegaly.

*James T. Wortham** (by invitation) and *E. C. Hamblen*.

From the Division of Endocrinology, Duke Hospital and Duke University School of Medicine, Durham, N. C.

A patient with the syndrome of polyostotic fibrous dysplasia of mild degree and unilateral distribution, precocious puberty and minimal pigmentation showed acromegalic changes beginning at age 26. Her course was followed for seven years. The acral changes remained progressive despite high-dosage roentgen therapy to the pituitary. Serum phosphorus levels used as an index of growth hormone activity remained high. There was no evidence of sellar destruction, optic impingement or increased intracranial pressure.

There are highly varied and numerous endocrine components to the collected cases of Albright's syndrome. Precocious release of FSH; precocious release of LH; excessive production of TSH; and now, excessive production of growth hormone; all have occurred by implication in association with the bone and skin lesions described.

The common mediator of these endocrine changes may be the variable but almost inevitable hyperostosis of the base of the skull in these patients. Through a variety of mechanisms, this hyperostosis may cause impairment of normal function of neurologic areas in the vicinity of the floor of the third ventricle. These areas, by inference, play a large part in control of pituitary tropin secretion. Acromegaly has previously been seen in association with hypothalamic changes and without pituitary neoplasms. These neuroendocrine relationships are, however, largely conjectural.

134. The Reciprocal Effects of TSH and Thyroxine Upon the Iodine Concentrating Capacity of Rat Thyroids.

Sidney H. Ingbar and *John Roberts* (introduced by Laurence H. Kyle).

From the Department of Biophysics, Army Medical Service Graduate School, Walter Reed Army Medical Center, Washington, D. C.

The influence of TSH on the capacity of the thyroid gland to concentrate inorganic iodide was assessed in both intact and hypophysectomized rats in which this mechanism was isolated from subsequent steps in hormone synthesis by means of dietary propylthiouracil. Prior to the administration of a standard dose of radioactive iodide, animals were maintained on varying doses of thyroxine, TSH, or both. Animals were sacrified serially, measurements made of inorganic I^{131} concentrations in the plasmas and thyroids, and concentration gradients calculated (inorganic I^{131} per gram thyroid/inorganic I^{131} per ml. plasma). Concentrations of organically-bound I^{131} in the thyroids of all groups were negligible, indicating that a complete block of hormone synthesis had been achieved. TSH, in total dosage graded from .0075 to 0.4 mg. (3–4 JSU/mg.), increased the iodide-concentrating capacity of hypophysectomized rats and of intact rats given 20 mcg. of thyroxine daily in order to suppress endogenous TSH secretion. Larger doses of TSH produced no significantly greater effect. Although control concentration gradients in hypophysectomized animals were from 8 to 10 times as great as in thyroxine-treated intact animals, the gradients of both groups were enhanced 25-fold by maximally effec-

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tive doses of TSH. This augmentation by TSH was diminished in those hypophysectomized animals concurrently given thyroxine. The data indicate a reciprocal influence of TSH and thyroxine upon the thyroidal iodide-concentrating mechanism.

135. The Metabolic Activity of 2′,6′Diiodothyronine, an Analogue of Thyroxine.

Charles A. Plamondon (by invitation), *John G. Wiswell* (by invitation) and *Samuel P. Asper, Jr.*

From the Department of Medicine, Johns Hopkins University, School of Medicine, Baltimore, Md.

Altering thyroxine (3,5,3′,5′ tetraiodothyronine) by removing some of the iodine atoms or by substituting other halogens or nitro groups for iodine has resulted in compounds which retain, although to lessened degree, calorigenic activity. However, Cortell (*J. Clin. Endocrinol.* **9**: 55, 1949) found the thyroxine analogue, 2′6′ diiodothyronine, to have no thyroxine-like and definite antithyroxine activity, by determining its effect on thiouracil-induced thyroid hypertrophy in rats. Studies of the calorigenic activity of this analogue comprise this report. In contrast to thyroxine, no acceleration of O_2 consumption occurred from single doses of analogue in normal and hypothyroid rats at molar ratios to thyroxine from 200 to 500, nor did the analogue prevent the rise of O_2 consumption by thyroxine. Four groups of hypothyroid rats received daily injections of saline, thyroxine, analogue, or both thyroxine and analogue; at seven and fourteen days, O_2 consumption was accelerated in the thyroxine group, unchanged in the saline and analogue groups, and variable in the group receiving both drugs. Normal rats receiving analogue daily had no alteration of O_2 consumption. O_2 consumption of isolated liver slices from normal and hypothyroid rats receiving analogue was unaffected. That the analogue lacks calorigenic effect adds further evidence attesting the importance of the 3,5 positions of the thyronine molecule in metabolic activity.

136. The Effect of Goitrogen Withdrawal on Thyroidal I^{131} Uptake in the Rat.

Savino A. D'Angelo, Karl E. Paschkis, Abraham Cantarow, Albert N. Siegel (by invitation) and *Gladys Friedler* (by invitation).

From Jefferson Medical College, Philadelphia, Pa.

Depressed thyroidal I^{131} uptakes in goitrous guinea pigs rebound substantially above normal following withdrawal of goitrogen (D'Angelo *et al.*, *Endocrinology* **49**: 624, 1951). The present study was designed to determine whether the "rebound" phenomenon exists in the rat also. Groups of adult female rats, varying as to duration of treatment with propylthiouracil (0.05 per cent in ration, for from ten to seventy-two days), were removed from the drug regimen and maintained on a normal diet until time of sacrifice (from two to thirty days following drug withdrawal). Twenty-four hours before sacrifice, these animals, together with appropriate control groups, received a tracer dose of I^{131} (4 microcuries, without carrier). Radioactivity measurements were subsequently made on the right thyroid lobe after homogenization in NaOH. It was found that thyroidal radioiodine uptakes in rats treated for from thirty-four to seventy-two days remained depressed for at least twenty-eight days following drug withdrawal. For shorter periods of goitrogen administration (twenty days), I^{131} uptake (per mg. of wet thyroid tissue) was still subnormal from three to fifteen days after withdrawal, but total thyroidal radioiodine collections approximated normal. Drug withdrawal after ten days of treatment resulted in augmented avidity of thyroid tissue for radioiodine, with total radioactivity of the gland increasing 50 to 100 per cent above normal. The results indicate

that goitrogen withdrawal in the rat, like the guinea pig, induces the rebound phenomenon of thyroidal radioiodine uptake. The time relationships involved, however, differ considerably for these rodents.

137. Changes in the Circulating and Bone Marrow Eosinophils Due to the Action of Thiouracil in Hyperthyroid and in Normal Subjects.

F. A. de la Balze, R. Celani Barry (by invitation), *F. C. Arrillaga* (by invitation) and *R. E. Mancini* (by invitation).

III Catedra de Clinica Medica, University of Buenos Aires, Buenos Aires, Argentina.

The variations of the circulating eosinophils and the eosinophil mother cells of the sternal bone marrow, which appeared when methylthiouracil or propylthiouracil was administered to 21 hyperthyroid patients and 9 normal subjects, were studied.

1) The most noteworthy modification was the disappearance of the circulating eosinophils, not accompanied by significant modification of the remaining leukocytal formula. This occurred in 6 hyperthyroid and in 1 euthyroid subject. The bone marrow was studied in 6 of these subjects, and in 4 there was absence of eosinophil precursors but there was no marked alteration of the remaining leukocyte mother cells. These modifications are reversible, since with withdrawal or reduction of thiouracil intake, eosinophils reappeared in the blood in 5 of 6 cases and there was a return of the sternal bone marrow to normal.

2) Four patients (13.3 per cent) showed a manifest decrease in circulating eosinophils which, however, did not disappear entirely.

3) In 5 patients (16.6 per cent) a significant rise in circulating eosinophils was observed.

4) Fourteen patients (46.6 per cent) did not show any significant modifications.

5) The action of thiouracil on the circulating eosinophils and the eosinophil precursors of the bone marrow seems to be a precocious and selective phenomenon.

138. A Simplified Technique for the Diagnosis of Hyperthyroidism Utilizing the One-hour Uptake of Orally Administered I^{131}.

K. R. Crispell, William Parson and *Philip Sprinkle.*

From the Dept. of Internal Medicine, University of Virginia, Charlottesville, Va.

The twenty-four-hour uptake of radioactive iodine, I^{131}, in the thyroid has received acceptance as an aid in the diagnosis of hyperthyroidism. This study was undertaken to determine if the time interval between ingestion and uptake could be shortened without sacrificing accuracy. This would then make it a more useful test.

Sixty cases referred for thyroid evaluation received an oral tracer dose (4 to 8 microcuries) of radioactive iodine in the fasting state, and the uptake in the thyroid was measured by means of a scintillating counter. The uptake was determined one hour and twenty-four hours after ingestion. Nineteen of these patients, proved to be hyperthyroid and had a one-hour uptake ranging from 23 to 57 per cent (41 euthyroid subjects showed 5 to 19 per cent). The twenty-four-hour uptake ranged from 46 to 98 per cent (euthyroid, 13 to 45 per cent). Similar results were obtained in 45 subjects (11 of whom were hyperthyroid) who were given an intravenous injection of radioactive iodine as recommended by Kriss.

The one-hour uptake after orally administered I^{131} is effective in differentiating the hyperthyroid from the euthyroid state. The advantages include simplicity of administration and reduction of time.

139. Paraoxipropiophenone in the Treatment of Parry-Graves-Basedow Syndrome and in "Pure Hyperthyroidism"—Clinical Report of 12 Cases.

J. Chavez Montes.

Universidad Nacional de Mexico, Mexico City, D. F.

Paraoxipropiophenone has been used by us for the first time in Mexico and perhaps in Latin America for the treatment of the Parry-Graves-Basedow syndrome and in "pure hyperthyroidism."

Twelve cases are reported: 5 Basedowians, 5 of pure hyperthyroidism, 1 of hyperthyroidism with unilateral exophthalmos, and 1 of toxic nodular goiter.

The 5 Basedowians demonstrated a great improvement of the hyperthyroid picture, disappearance of the oculo-facial assynergies and considerable regression of exophthalmos in 2, discrete in 2 more, and unmeasurable in 1. Pure hyperthyroid patients showed great improvement of the general condition, with lowering or even normalization of the basal metabolism, increase of the blood cholesterol level and lowering of the blood iodine levels; as for oculo-facial assynergies, improvement or complete disappearance was achieved in all cases. Frenantol showed itself useless in a case of nodular goiter. All symptoms, with the exception of exophthalmos, were controlled in 1 case of unilateral exophthalmos. Tolerance was excellent in all cases.

140. Further Studies on Tissue Responses to Thyroxine.

S. B. Barker and *H. S. Schwartz* (by invitation).

From the Department of Zoology, University of California at Los Angeles, California.

It has previously been found that thyroxine injected into adult albino rats resulted in an increased oxygen consumption from liver, kidney, diaphragm, heart, skeletal muscle, gastric mucosa and gastric smooth muscle. In contrast, brain, spleen and testis showed no oxidative responses.

Knowledge of the responses of various tissues to thyroid hormone has been extended to salivary gland and to pancreas, which exhibited QO_2 increases of 30 and 45 per cent, respectively, to doses of thyroxine which elevated liver, kidney and diaphragm 40 to 60 per cent. In the same animals, changes of less than 10 per cent were shown by thymus, lymph nodes, seminal vesicle, prostate, ovary and uterus. (Acknowledgment is made to the Krichesky-Ponty Fellowship for support of this work.)

141. Quantitative Determination of the Steroid Hormones, Using 2:4-Dinitrophenylhydrazine.

A. G. Gornall and *M. P. Macdonald* (introduced by R. F. Farquharson).

From the University of Toronto, Toronto, Canada.

Phenylhydrazine was used by Porter and Silber (*J. Biol. Chem.* **185:** 201, 1950) in a colorimetric procedure for 17,21-dihydroxy-20-ketosteroids. Because this method was found unsuitable for urine extracts, conditions were defined under which the reaction with 2:4-dinitrophenylhydrazine could be made the basis of a sensitive quantitative procedure (Gornall and Macdonald: *Proc. Can. Physiol. Soc.* p. 18, 1951). The assay is carried out on 2–15 μg. of corticosteroid and, rating the optical density produced by cortisone as 100 per cent, the reactivity of the various groups on an equimolar basis has been assessed as follows: A ketone in a saturated ring (3–6 per cent); C-3 ketone with ocβ unsaturated bond (42 per cent); C-20 ketone (3 per cent); C-20 ketone, C-17hydroxyl

(12 per cent); C-20 ketone, C-21 hydroxyl (20 per cent); C-20 ketone, C-17, C-21 dihydroxyl (54 per cent). The reaction with the $\alpha\beta$ unsaturated C-3 ketone proceeds rapidly and quantitatively in about ninety seconds at room temperature with less than 10 per cent interference from other groups. The reaction with the C-20 ketone in an hydroxylated side chain is slower and not quite complete even after ninety minutes at 58°C. Because of this difference it is possible to estimate selectively the C-3 ketone alone or along with the ketol side chain. Some useful applications of the method are reported.

142. The Effect of Adrenocorticotropic Hormone on Blood Glucose and Insulin Hypoglycemia.

Saul B. Appel (by invitation), *Joseph L. Gluck* (by invitation), *Stanley Reichman* (by invitation), *Austin A. Schlecker* (by invitation), *Alfred Miller* (by invitation), *Abraham M. Goldman* (by invitation) and *Herbert S. Kupperman.*

From the Third (New York University) Medical Division, Goldwater Memorial Hospital, New York, N. Y.

The influence of intravenous infusion of ACTH upon the fasting blood glucose and upon the hypoglycemic response to intravenous insulin was determined in patients demonstrating normal glucose and insulin tolerance. Eosinophil response and urinary glucose were determined. The eosinophil response was not increased by ACTH doses greater than 12.5 mg. ACTH infusion of four hours' duration produced significant hyperglycemia with a 30 per cent elevation in blood sugar at the 25-mg. dose level. No greater elevation occurred despite dosage increments to 100 mg. of ACTH. No glycosuria was noted. Intravenous infusion of ACTH failed to induce hyperglycemia in an adrenalectomized patient. A definite diminution occurred in the degree of insulin hypoglycemia when ACTH was infused for four hours prior to insulin injection, with greatest effect obtained at the 50-mg. dose level. The diminution in hypoglycemia decreased progressively with higher dosage of ACTH. No diminution in insulin-induced hypoglycemia was noted after two-hour infusions of ACTH. Dosage-response curves were constructed for these functions of ACTH activity. The possible use of such data for human bio-assay is discussed.

The Endocrine Society

FIFTH ANNUAL POSTGRADUATE ASSEMBLY IN ENDOCRINOLOGY AND METABOLISM

Sponsored by

THE ENDOCRINE SOCIETY

Roney Plaza Hotel

Miami Beach, Florida *November 3–8, 1952*

The faculty will consist of 20 clinicians and investigators eminently qualified in the fields of endocrinology and metabolism.

The program will consist of lectures, clinics, laboratory demonstrations and scientific exhibits.

The 64 lectures to be presented will cover all phases of endocrinology and metabolic disorders. Ample time will be given for discussions and answers at the end of each session and registrants are encouraged to contact members of the faculty for individual discussions. The entire program has been designed to interest not only the specialist but those in general practice and has been approved by the American Academy of General Practice for postgraduate credit.

A simultaneous translation of all lectures into Spanish will be provided through earphones.

A copy of the proceedings of the Assembly will be available to all registrants.

The Roney Plaza with its tropical gardens, outdoor swimming pool, putting greens, private beach and Cabana Club, has long been recognized as one of Florida's great beach hotels and offers special convention rates to members of this Assembly. The Surf Room, in which the sessions will be held, is delightfully air conditioned and has subdued lighting which permits taking of notes and viewing lantern slides with comfort.

A fee of $75 will be charged for the entire course and the attendance will be limited to 200. *Registration will be in the order of checks received and will close on October 3, 1952.* Should there be an insufficient number of applicants to fill the course, the registration fee will be refunded immediately in its full amount.

Please forward application on your letterhead, together with check payable to The Endocrine Society, to Henry H. Turner, M.D., Secretary-

Treasurer, 1200 N. Walker Street, Oklahoma City 3, before October 3, 1952. Further information and program will be furnished upon request.

Hotel reservations should be made directly with the Roney Plaza Hotel, Miami Beach, Florida, and the hotel advised that you are attending this Postgraduate Assembly.

OBITUARY

25, 1952

DEAR SIRS,

We regret to announce to you the death of

DR. ELISABETH DINGEMANSE

at the age of 65 years.

As you will know, Dr. Dingemanse was one of the co-workers of the late Professor Dr. Ernst Laqueur.

In the early years of insulin manufacture, she made substantial contributions to the purification of this hormonal preparation. She played a major part in the chemical work on female hormones; the isolation of the male hormone was achieved with her help in the pharmacological laboratory in Amsterdam.

In later years she became world-famous by her method on the quantitative and qualitative estimation of the 17-ketosteroids.

The Municipal University of Amsterdam honoured her by conferring upon her a doctor's degree of medicine.

For many years Organon enjoyed a close collaboration with Dr. Dingemanse and those who knew her personally will always remember her for her highly scientific qualities, her modesty, her readiness to help and her kindness.

N. V. ORGANON

DR. J. W. R. EVERSE, *Medical Director*

JCM

UNITED STATES ATOMIC ENERGY COMMISSION

CANCER PROGRAM FOR FISCAL YEAR 1953

The U. S. Atomic Energy Commission, in fiscal year 1953, is changing its previous policy of distributing radioisotopes for cancer research and therapy free of production costs. Instead, it will make a charge of 20 per cent of production costs. This change in policy affects only the price, and not the availability of isotopes; production will continue to meet all demands.

In 1948 the Atomic Energy Commission initiated a balanced program for utilization of its special facilities and knowledge in the field of cancer research. As part of this program, radioactive sodium, phosphorus and iodine were made available free of production costs for use in the study, diagnosis and treatment of cancer and allied diseases. The following year the program was enlarged to include other radioisotopes distributed by the Commission, provided the materials were to be used in cancer studies or treatment.

The field has developed so rapidly that certain clinical applications of radioisotopes now have become a matter of routine, and research workers have become fully aware of the usefulness of these atomic tools in cancer research. The Commission's Advisory Committee for Biology and Medicine and Advisory Committee on Isotopes Distribution recently have advised that the stimulus of completely free distribution no longer is necessary to encourage the use of radioisotopes in the field of cancer. These committees have agreed with the view of the AEC that a charge of 20 per cent of catalog prices will not hamper advances in this field, and that it will help to indicate the future demand for radioisotopes in relation to other tools for cancer research and therapy.

Radioisotopes are being used in the study of a number of other diseases, such as heart disease and hyperthyroidism, as well as in the study of cancer. However, the full production cost has been charged for radioisotopes used in studies of diseases other than cancer.

The following table shows the prices per millicurie which will be charged, beginning July 1, 1952, for the four most important radioisotopes used in cancer research or therapy, compared with prices for non-cancer use:

	Cancer Use	*Non-Cancer Use*
Radioiodine 131	$0.15	$0.75
Radiophosphorus 32	0.22	1.10
Radiogold 198	0.05	0.24
Radiocarbon 14	7.20	36.00

Application forms may be procured from the Isotopes Division, Atomic Energy Commission, Oak Ridge, Tennessee.

UNIVERSITY OF MINNESOTA SYMPOSIUM ON THE METABOLISM OF POTASSIUM

Sponsored by the

M AND R LABORATORIES, INC., COLUMBUS, OHIO

To be Presented on the University of Minnesota Campus, Minneapolis
September 22, 23, and 24, 1952

IRVINE MCQUARRIE, M.D., Chairman

Experimental and clinical aspects of potassium metabolism will be presented by outstanding investigators from various medical centers. A partial list of the participants includes:

DR. ALLEN M. BUTLER	Boston, Massachusetts
DR. PAUL R. CANNON	Chicago, Illinois
DR. T. S. DANOWSKI	Pittsburgh, Pennsylvania
DR. DANIEL C. DARROW	New Haven, Connecticut
DR. WALLACE O. FENN	Rochester, New York
DR. RICHARD H. FOLLIS	Baltimore, Maryland
DR. GEORGE M. GUEST	Cincinnati, Ohio
DR. JOHN E. HOWARD	Baltimore, Maryland
DR. LAURANCE W. KINSELL	Oakland, California
DR. GILBERT H. MUDGE	New York, New York
DR. JOHN P. PETERS	New Haven, Connecticut
DR. ROBERT TARAIL	Pittsburgh, Pennsylvania

Members of the University faculty, including the Mayo Foundation, will also participate.

The detailed program will be announced at a later date. Sessions of the Symposium will be held either in the Auditorium of the Museum of Natural History or in Northrop Memorial Auditorium. Living quarters will be available at moderate cost in the dormitories of the University. Members of the medical and allied professions are invited. There will be no tuition fee.

For further information, write to the Director of Continuation Medical Education, University of Minnesota Hospitals, Minneapolis 14, Minnesota.

HARVARD MEDICAL SCHOOL POSTGRADUATE COURSE IN PEDIATRIC ENDOCRINOLOGY AND METABOLISM

A Postgraduate Pediatric Endocrinology and Metabolism Course will be held at the Burnham Memorial Hospital for Children, Massachusetts General Hospital, Boston, under the direction of Dr. Nathan B. Talbot and associates, October 6 through October 11, 1952, daily from 9:00 A.M. to 4:30 P.M. For further details, write Courses for Graduates, Harvard Medical School, 25 Shattuck Street, Boston, Mass.